Providing better care to HIV-infected pregnant women, children and their families in a rural sub-Saharan African clinic

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

von

Anna Gamell

aus Spanien

Basel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Prof. Dr. Marcel Tanner, Prof. Dr. Manuel Battegay und Prof. Dr. David Nadal.

Basel, den 20. Juni 2017

Prof. Dr. M. Spiess

Dekan der
Philosophisch-Naturwissenschaftlichen Fakultät

To all children and adolescents living and growing with HIV, especially to those I have met. They have given me more than I will ever be able to give back.

Kwa watoto wote wanaoishi na kukua wakiwa na maambukizi ya VVU, na zaidi sana wale ambao nimekutana nao. Wamenisaidia kwa kiasi kikubwa ambacho sitaweza kuwalipa.

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Acknowledgements

This thesis is the result of the work done in Ifakara during the last five years. I have only been able to complete it thanks to the many people that I have met and who have stood by, helped, supported and collaborated with me. I dedicate this thesis to all these people.

My interest for paediatric HIV was born during my first stay in Africa. Few weeks after finishing medical school I travelled to Manhiça, Moçambique, for a six months stay. During those months I realized how much HIV/AIDS affected the health and lives of many children and their families in sub-Saharan Africa. I have to thank in first place Pedro Alonso, who made that stay possible, and the Centro de Investigação em Saúde de Manhiça, that welcomed me. Quique Bassat was the paediatrician working there at the time; he was an inspiration for some of the steps that followed that stay, gràcies. While in Manhiça I met some people who are now good friends: Tali, Salut, Sergi and Jaume. To Telma and Lucinha I have to thank their openness and their efforts to show me the daily life in Manhiça. Emili was also in Manhiça, it was there where our "us" started, but he deserves a full paragraph and it comes later.

After Manhiça I had it clear: I wanted to become a paediatrician and I wanted to work with children living with HIV in sub-Saharan Africa. I did my paediatrics residency at Hospital Sant Joan de Déu in Barcelona. My residency's tutor, Gemma Claret, was amazing. She was always there, never tried to change what I wanted, and instead, gave me good advice, guided me and provide me with the necessary support and tools to finish the training and follow my way. I am also grateful to Clàudia Fortuny and Vicky Fumadó, both paediatricians that inspire me and with whom I have the honour to work now. From the four years of the residency and the many days and nights spent in the hospital, I will always remember the good times spend with my peers: Patri, Asier, Luciana, Núria, Angi, Míriam, Marta, Xavi, Cris, Sílvia and Paco.

After finishing the residency I was given the opportunity to work for the Swiss TPH in Ifakara, Tanzania. There is no doubt of who I have to thank for this: Marcel Tanner and Christoph Hatz. I will always remember my first visit to Basel and the interview with Marcel. I was, and I still am, impressed by his character, open mind, vision and love for Ifakara. For all this and many more, thanks. During that same visit, I met Christoph. He and Christine took Emili and me for dinner. By the Rhine I felt the passion Christoph has for the clinical work, a passion that I share and that I appreciate the most from a boss. Thanks Christoph.

Later on I met Manuel Battegay. The supervisor of this PhD. Few lines here will not be enough to thank you for the supervision, great ideas and better advices during the last years. And if I think of Manuel, Muriel comes to my mind. Muriel, thanks for taking care of me during my stay in Basel last year.

In 2012, we moved to Ifakara. The work included in this thesis started. I would like to thank the Ifakara Health Institute and its former and current directors, Salim Abdula and Honoraty Masanja, for having welcomed me and supported this work. A very special thanks goes for Frederick Masanja, the branch leader in Ifakara and our neighbour for three years. He was excellent in both roles.

The Chronic Diseases Clinic of Ifakara is where I worked. I feel an enormous love for this clinic and for all people that work there and make it what it is: a clinic where people living with HIV receive respectful and quality health care. I am grateful to all the staff for how they received me in the first place, for all that I learnt from them, and for the thousands of hours we shared working, and for some of these hours being full of laughs. Asanteni sana! My deepest thanks are for the One Stop Clinic team. To Dolores and Mama Chale for being there from the very first day, for being the most wonderful nurses to provide care to children and mothers in Ifakara, and for having treated me as her daughter, even feeding me during the years in Ifakara! Mama Chale and Dolores: ninashukuru sana! Leila joined the team a little later, and what a change! With her the One Stop Clinic boosted and the psychological support that pregnant women, children and caregivers receive since then has only improved their outcomes. Thanks Leila. And now the clinicians: Lameck, Ezekiel, Getrud and Lilian. Working side by side with you has been an honour. I deeply appreciate the passion you feel for your work and I can only say asante, asante, asante, asante!

Lukas Muri deserves a special acknowledgement. Without him two of the studies included in this thesis would not have been possible. The techniques you implemented together with the lab team in Ifakara represented a real change. Since then, the care that patients, and especially young infants, receive is much better. Thanks for your generosity.

The clinic in Ifakara would not be possible without the work of a group of people that from Switzerland, dedicate an important part of their time to support the clinic. Without them this thesis and many other projects could not have happened. Thanks Tracy, Thomas, Ingrid, Fiona and Tschabi.

The infants, children and adolescents who attend the One Stop Clinic deserve the biggest thanks. They are the reason why the job as paediatrician during the four years I lived in Ifakara is the best clinical work I have ever had. From the tinny fatty babies to the oldest adolescents, you are the best patients a paediatrician can dream of. Visiting you, seeing you growing, and answering your doubts made me happy every single day, no matter what other things were happening. Now, from Europe, I miss you and I do not forget the ones we could not help enough. To the mothers, fathers, bibis and caregivers of these kids, thanks for trusting us.

Life in Ifakara was not only about work. I met fabulous people that became good friends and who were my family far from home. Together we shared beers, canoe trips, safaris, camping weekends, dinners, Christmas parties and much more. Polly, Gerry, Eleanor, Lyam, Lauren, Mamiko, Maria, Jesse, Jason, Kathy, Morgan, Kate, Tevita, Magnus, mama Emili, little Emili, Said, Jessica, Lena, Stefan, Lukas, Herry, George, Irene, Birgit, Maja, Martin and Johan, thanks a lot.

After I came back from Tanzania, I spend some months in Basel. Those few months rounded the PhD, I enjoyed each lecture and met fantastic people with whom I would have continued to share office, evenings and train trips for much longer. Hala, Shala, Josephine, Wyvine and Chrsitian, thanks! I cannot thank enough Christine Walliser, Christine Mensch and Margrith Slaoui. They help me every single time I ask them, and it is quite often. Thanks to all of you for your always kind and warm support.

This thesis is also dedicated to my friends from childhood and medical school, the ones that I have grown with and without whom I cannot imagine my life: to Monica, Cristina, Alba, Sandra, Mar, Anna, Laura, Mireia, Tamara, Jordi, Sònia and Josep Maria. Thanks for always being there.

I must thank my family. Although they were probably not quite convinced, my parents supported my first stay in Moçambique. Since then, they have always been by my side, no matter how far and for how long I leaved. Gràcies per això i per molt més. I will never find enough words to express how much I love my sister, and how much I feel loved by her. Gràcies Tata. Thanks also to Steve and Marc, for making my sister happy and for making our family grow. Thanks to my grandparents: avi Lluís, iaia Maria, avi Miquel and iaia Mercè. Each one of you, with your own way, have always been sweet and generous to us. Gràcies. Matete, Sun, Paul, Martina and Violeta are also part of my family. Thank you for having me as part of yours.

Emili, I have to thank you for an endless list of things. Your passion for medicine and for HIV/AIDS spreads to anyone working with you, and it also got me. It was you and your enthusiasm that made possible previously unimaginable changes at the Chronic Diseases Clinic of Ifakara. With you, the clinical care, training and research in Ifakara improved to a level that nobody expected. I have to thank you not only for supervising each single study included in this thesis, but also for making them happen, from the initial idea to the very last writing detail. It is now eleven years since we first lived together in Moçambique. I am grateful to life for making us met and build up our love story. Every chapter has been amazing and I am now excited about today and about what it is coming. Parenthood will be another great thing to share with you. Mil gràcies per això i moltíssimes altres coses. I dedicate this thesis to you and to our soon-to-be son. And to him, to our future son, I have to thank the energy that he has given me during these last months. Working with you inside has been an endless source of inspiration.

Finally, I have to thank the funders of the Chronic Disease Clinic of Ifakara and the R Geigy Foundation. A special thanks is for the Merck for Mothers Global Giving Program and Erik Mossdorf. The grant this program awarded us allowed the initial implementation of the One Stop Clinic and contributed to everything that has come with it.

Barcelona, August 2017

Summary

The HIV/AIDS pandemic has dramatically concentrated in sub-Saharan Africa. There are 36.7 million people living with HIV (PLWHIV) worldwide and over 50% of them reside in Eastern and Southern Africa. Sub-Saharan Africa is also home of 85% of pregnant women living with HIV and 90% of HIV-infected children. During the last decade prevention of mother-to-child transmission (PMTCT) programs have been implemented in most countries with an increasing coverage and an estimated reduction of new paediatric HIV infections by 60% since the year 2000.

Although HIV/AIDS appears to have stabilized worldwide, many challenges persist. Despite the progressive roll-out of PMTCT, in 2015, nearly a quarter of pregnant women living with HIV did not access antiretrovirals drugs and an estimated 150,000 new paediatric infections occurred. There are 1.8 million children living with HIV and they represent an underprivileged population. Importantly, the scale-up of paediatric ART has encountered substantial barriers and in 2015, only 49% of children in need accessed treatment.

Since the early 2000's different PMTCT recommendations have been developed for resource-limited countries. These recommendations have been periodically revised and updated based on the latest evidence and the implementation experiences. Currently, the WHO recommends what is known as PMTCT "Option B+": lifelong ART for all pregnant and breastfeeding women regardless of their CD4 counts and clinical stage. The main challenges that drove to these latest PMTCT guidelines were the unresolved health system barriers to CD4 testing for ART eligibility, the high fertility rates in countries most affected by HIV and the perceived need of simplification of recommendations. The supposed benefits of Option B+ were: a) streamlined implementation given its simplicity; b) high potential to reach maximum coverage and promote the virtual elimination of paediatric HIV; c) reduced HIV transmission to partners; and d) protection from conception of the subsequent pregnancies. While Option B+ has truly resulted in better ART coverage among pregnant women and in a decrease in the number of new paediatric HIV infections, some barriers to its optimal potential still exist. The efficiency of the guidelines simplification will only be fully palpable if the inadequate human resources, infrastructure and supply chain are addressed. Moreover, strategies to ameliorate the high early defaulter rate have to be prioritised. Adequate counselling and preparation of patients before initiating ART and patient education need to be put at the frontline along with other program areas to ensure adherence among mostly asymptomatic pregnant women and mothers.

Children infected with HIV present more rapid diseases progression than adults. Timely diagnosis and ART initiation is therefore vital for this population. Testing and diagnosing children for HIV must be enhanced within PMTCT programs, ensuring that all HIV-exposed infants are tested within the first two months of life and that results reach their caregivers. Also, approaches to identify HIV-infected children outside PMTCT programs must be expanded to places where children congregate, especially to places where children at high risk of HIV may seek for services. Yet, after being diagnosed with HIV, children encounter barriers to access life-saving treatment. These barriers are mainly related to the service delivery models, the health workers' capacity to provide quality paediatric HIV/AIDS care and treatment, and the availability of paediatric antiretroviral formulations.

Due to several factors, long-term treatment success and virological suppression is harder to achieve in children living with HIV compared to adults. The high rates of virological failure and development of acquired drug resistance-associated mutations, coupled with the limited paediatric antiretroviral drugs available, represent a threat to paediatric ART programs in Africa that needs attention.

Novel strategies to increase the uptake of PMTCT services are needed in sub-Saharan Africa to reach the goal of elimination of paediatric HIV. In this same region, HIV diagnosis, linkage into care, ART coverage and treatment outcomes of children need to be improved. Family-centred approaches may facilitate access to HIV and improve the clinical care provided to pregnant women, mothers and children living with HIV and their families.

This thesis was developed mainly as an operational and implementation science research. Through the work presented here we designed and tested a family-centred HIV service delivery model in a rural Tanzanian setting, Ifakara. The basis was the identification of gaps for delivering PMTCT in this rural setting. First, we conducted a cross-sectional study to assess the PMTCT services uptake in Ifakara. Second, we designed and implemented a package of measures as a strategy to improve paediatric and maternal HIV care. Third, we evaluated the impact of the strategy on the clinical outcomes of pregnant women and children by comparing prospectively collected data from before and after the implementation. Forth, we did a prospective study evaluating the PMTCT cascade after the implementation of the package of measures and the roll-out of Option B+ in the country and we compared the results with the initial cross-sectional assessment. Last, we assessed the outcomes of children and adolescents on ART and investigated the prevalence and determinants of virological failure and acquired antiretroviral drug resistances.

The first study assessed the PMTCT care pathway in Ifakara during the period 2010 – 2011. It described the departments and health workers involved and the circuit pregnant women, mothers and infants were supposed to follow, identified the existing gaps, and proposed solutions to bridge them and improve the outcomes of HIV-infected mothers and their offspring. Although all services and most resources were in place for a well-functioning PMTCT program, major gaps were identified, such as the very poor linkage to HIV care and the lack of a standardised follow-up for HIV-exposed infants. The study emphasised the need for much simpler and effective PMTCT recommendations, as Option B+, a new model of care integrating maternal and HIV services, and an update of the training that health workers attending HIV-infected pregnant women received. This first study set the basis for the development of the One Stop Clinic, the maternal and paediatric unit of the HIV clinic of Ifakara, a unit designed to be integrated within the reproductive and child health clinic.

The second and third studies assessed the impact of the One Stop Clinic on the care provided to HIV-infected mothers, children and their families. Study 2 described the baseline characteristics, clinical outcomes and retention in care of pregnant women and children enrolled in HIV care before (2008 – 2012) and after (2013 – 2014) the implementation of the One Stop Clinic in combination with the evolution to a paperless clinic and the expansion of provider-initiated HIV testing and counselling in the hospital wards. The strategy resulted in an increased number of mothers and children diagnosed and linked into care, a higher detection of children with AIDS, universal treatment coverage, lower loss to follow-up, and an early mother-to-child transmission (MTCT) rate below the threshold of elimination. Hence, this study documents a feasible and scalable model for family-centred HIV care in sub-Saharan Africa.

The third study re-assessed the PMTCT cascade in Ifakara during the period 2014 - 2015. It evaluated the impact of the measures taken after the first assessment presented in Study 1 combined with the adoption of Option B+ guidelines by the Government of Tanzania. PMTCT was assessed at the antenatal clinic, HIV care and labour ward, and compared with the pre-B+ period. The implementation of Option B+ through the One Stop Clinic model resulted in universal HIV testing in the antenatal clinic, high rates of linkage to care and ART provision, and a MTCT rate of 2.2%. However, HIV testing in late pregnancy and labour were poor, and retention during early ART was not optimal. This study reports the improvements reached with Option B+ implemented through a comprehensive service delivery model and drives attention to the barriers limiting the full efficacy of Option B+ recommendations.

The forth study presented a well-characterised series of HIV-infected children from rural Tanzania with virological failure and acquired drug resistance-associated mutations. The acquisition of resistance mutations among African children living with HIV has been scarcely reported and this study contributes to the better understanding of this emerging public health concern.

The results of Study 4 drove the attention to an emerging concern that needed a more comprehensive characterization. This is the rationale for the fifth study. All children and adolescents attending the One Stop Clinic in Ifakara and on ART for at least twelve months were included in this study. Among 213 children and adolescents, 25.4% presented virological failure. ART-associated resistance mutations were indentified in 90% of them, with multiclass resistances in 79%. Suboptimal adherence, female gender, and current non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART independently increased the odds of virological failure. Higher CD4 cell percentage and older age at ART initiation were protective. The findings of this study provide relevant information for clinicians and health policy makers and raise concerns about the effectiveness of current paediatric ART programmes in sub-Saharan Africa. The results of the study advocate for the strengthening of adherence strategies, the development of new paediatric drug formulations, and the universal roll-out of routine viral load monitoring.

In conclusion, the studies included in this thesis show that with an operational approach, real changes can be implemented in a rural Tanzanian setting. The One Stop Clinic model resulted in more pregnant women living with HIV diagnosed and linked into care, over 90% of them receiving drugs for PMTCT, and one of the lowest attrition rates reported from rural Africa. The outcome of such PMTCT service provision improvements is a MTCT rate of HIV of only 2.2%. The new paediatric HIV case finding approaches that came along the One Stop Clinic allowed diagnosing and treating HIV-infected children that were previously not identified. Moreover, the child-friendly approach of our strategy helped to better understand and analyse treatment failure among children, one of the major barriers for paediatric ART programs success. The work in Ifakara documents a feasible and scalable model for maternal and paediatric HIV care that if extended to other sub-Saharan African settings can contribute to the goals of zero new infections among children, keep mothers in good health and close the paediatric treatment gap.

Muhtasari

Janga la UKIMWI limeathiri zaidi nchi za kusini mwa jangwa la Sahara. Duniani kote, watu milioni 36.7 wanaishi na maambukizi ya VVU, Zaidi ya 50% kati yao wanaishi katika nchi za Afrika mashariki na kusini. Asilimia 85% ya wanawake wajawazito wanaoishi na maambukizi ya vvu, na asilimia 90% ya watoto wenye maambukizi ya VVU wanaishi katika chi za kusini mwa jangwa la Sahara. Kwa muongo uliopita, programu nyingi za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto zimefanyika katika nchi hizi, na zimepelekea kupungua kwa maambukizi mapya ya VVU kwa watoto kwa takriban asilimia 60% tangu mwaka 2000.

Japokuwa janga la UKIMWI linaonekana kupungua duniani kote, bado kuna changamoto nyingi. Pamoja na kuenea kwa programu ya kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto (PMTCT), kwa mwaka 2015 karibu robo ya wanawake wajawazito waishio na maambukizi ya VVU hawakupata dawa za kupunguza makali ya virusi (ARV) na kulikuwa na maambukizi mapya ya VVU kwa watoto 150,000. Watoto milioni 1.8 wanaishi na maambukizi ya VVU na wanawakilisha jamii zinazoishi katika mazingira magumu. Zaidi ya yote, usambazaji wa dawa za ARV za watoto unakumbwa na changamoto nyingi, na kwa mwaka 2015, ni asilimia 49% tu ya watoto wenye maambukizi walipata dawa za hizo za kupunguza makali ya maambukizi.

Tangu mwanzoni mwa miaka ya 2000, kumekuwa na mapendekezo tofauti ya kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto kwa ajili ya kutumika katika nchi zinazoendelea. Mapendekezo haya yamekuwa yakipitiwa na kurekebishwa kulingana na ushahidi wa kisayansi na matokeo ya utekelezaji. Kwa sasa shirika la afya duniani linapendekeza njia inayotambulika kama “OPTION B +”: Mwanamke mjamzito na anayenyonyesha atatumia dawa za ARV kwa maisha yake yote bila kujali kiasi cha seli zake za kinga (CD4) au hatua ya ugonjwa wa UKIMWI. Changamoto kubwa zilizopelekea mapendekezo haya ya sasa ni vikwazo katika upimaji wa seli za kinga (CD4) ili kutambua uhitaji wa matibabu ya ARV, kasi ya kuzaliana kuwa juu katika nchi hizi zilizoathirika Zaidi na UKIMWI, na kuonekana kwa uhitaji wa kurahisisha utekelezaji wa mapendekezo hayo. Faida zilizotarajiwa kutokana na matumizi ya “option B+” ni a) utekelezaji sawia kutokana na urahisi wake b) uwezekano wa kufikia watu wengi na kuondoa maambukizi kutoka kwa mama kwenda kwa mtoto c) Kupunguza maambukizi ya VVU kati ya watu walio katika mahusiano ya kimapenzi d) kuzuia maambukizi kwa watoto katika mimba zitakazofuata.

Japokuwa Option B+ imepelekea ongezeko la wanawake wajawazito walio katika matibabu ya ARV na kupungua maambukizi mapya ya VVU kwa watoto bado kumekuwa na changamoto zinazozuia utimilifu wake. Urahisi wa muongozo huu utathibitika zaidi iwapo changamoto za rasilimali watu, upatikanaji na usambazaji vitendea kazi na zitatatuliwa. Zaidi ya yote, hatua za kupunguza ongezeko la watu wanaokatisha matibabu zanatakiwa kupewa kipaumbele. Ushauri nasaha na maandalizi ya wagonjwa kabla ya kuanzisha dawa za ARV, na elimu ya UKIMWI kwa wagonjwa vinatakiwa kupewa kipaumbele sambamba na huduma nyingine ili kuongeza uzingatiaji wa matibabu kwa wanawake wajawazito na wanaonyonyesha wasio na dalili zozote za ugonjwa wa UKIMWI.

Watoto walio na maambukizi ya VVU wanapata dalili za ugonjwa wa UKIMWI mapema zaidi kuliko watu wazima. Kutambua maambukizi na kuanza dawa kwa wakati ni muhimu sana kwa watoto. Kupima na kutambua maambukizi ya VVU kwa watoto kunatakiwa kuwa kipengele katika programu za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto, kuhakikisha kwamba watoto wote waliozaliwa na wanawake wenye maambukizi wanapimwa katika miezi miwili ya kwanza ya maisha na majibu ya vipimo yanawafikia wazazi/walezi wao. Njia za kutambua watoto walio na maambukizi ya VVU nje ya programu za kuzuia maambukizi kutoka kwa mama kwenda kwa mtoto zinatakiwa kuimarishwa pia na kuongezwa katika maeneo wanapokusanyika watoto, hususan maeneo ambapo watoto wenye uwezekano mkubwa wa kuwa na mambukizi wanapatiwa huduma za afya. Hata baada ya kugundulika na maambukizi ya VVU, kuna changamoto nyingi katika kuwapatia watoto hawa matibabu yanayoweza kuokoa maisha yao. Changamoto hizi zinahusiana zaidi na mifumo ya utoaji wa huduma za afya, uwezo wa wahudumu wa afya kutoa huduma na matibabu stahiki kwa watoto waishio na maambukizi ya VVU na upatikanaji wa dawa za ARV zilizo katika mfumo na kiwango stahiki kwa watoto.

Kutokana na sababu mbalimbali, matokeo mazuri ya matibabu na kunyima virusi uwezo wa kuzaliana ni ngumu zaidi kufikiwa kwa watoto waishio na maambukizi ukilinganisha na watu wazima. Uwezekano mkubwa wa virusi kuendelea kuzaliana kwa kasi na kupata usugu wa dawa ukichanganywa na changamoto za upatikanaji wa dawa za VVU za watoto vinapelekea tishio kwa programu za huduma za ARV kwa watoto katika bara la Afrika, na vinahitaji kuangaliwa kwa ukaribu.

Hatua madhubuti za kuongeza upatikanaji wa huduma za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto zinahitajika katika nchi za kusini mwa jangwa la Sahara ili kufikia lengo la kutokomeza kabisa tatizo la Ukimwi kwa mtoto. Katika nchi hizi, Upimaji wa

VVU, kuanza huduma za matibabu, upatikanaji wa dawa za ARV na matokeo ya matibabu kwa watoto vinahitaji kuimarishwa. Programu zinazolenga familia nzima zinaweza kuongeza upatikanaji wa huduma na kuimarisha matibabu yanayotolewa kwa wanawake wajawazito, wamama na watoto waishio na maambukizi ya VVU pamoja na familia zao.

Utafiti huu ulijikita Zaidi katika utekelezaji wa huduma za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto. Kupitia mrejesho huu wa kazi, tulibuni na kujaribu utoaji wa huduma uanolenga familia kwa ujumla katika maeneo ya Tanzania vijijini, Ifakara. Lengo hasa lilikuwa kutambua changamoto katika utoaji wa huduma za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto katika maeneo ya vijijini.

Kwanza tulifanya utafiti kutambua mapokeo ya huduma za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto katika eneo la Ifakara. Pili tukabuni na kutekeleza mfumo wa huduma zinazolenga kuboresha huduma za afya kwa wanawake na watoto walio na maambukizi ya VVU. Tatu, tukafanya tathmini ya matokeo ya mfumo huu wa huduma katika afya za wanawake wajawazito na watoto kwa kulinganisha takwimu zilizokusanywa kabla na baada ya utekelezaji wa mfumo huu. Nne, Tulifanya tathmini ya huduma za kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto baada ya utekelezaji wa mfumo huu uliobuniwa sambamba na kuenea kwa huduma za Option B+ nchini, na kulinganisha takwimu za tathmini hii na zile za utafiti wa kwanza. Na mwisho tulifanya tathmini kwa watoto na vijana wanaotumia dawa za ARV ili kutambua ukubwa wa tatizo la kupata usugu wa dawa na virusi kuendelea kuzaliana mwilini na visababishi vyake.

Utafiti wa kwanza ulichunguza utekelezaji wa huduma za kuzuia maambukizi kutoka kwa mama kwenda kwa mtoto katika eneo la Ifakara kwa kipindi cha mwaka 2010 hadi 2011. Ulichunguza na kupambanua kwa undani kuhusu idara na wafanyakazi wanaohusika na utaratibu ambao mama wajawazito na watoto walitakiwa kupitia ili kupata huduma, ilitambua mapungufu yaliyopo na kupendekeza hatua za kuchukua ili kuboresha huduma za afya kwa wanawake wajawazito wenye maambukizi ya VVU na watoto wao. Japo kuwa huduma na miundo mbinu ya kuwezesha kutekelezaji wa programu za kuzuia maambukizi kutoka kwa mama kwenda kwa mtoto ilikuwepo, baadhi ya vikwazo viliibuliwa ikiwemo utaratibu hafifu wa kuwaunganisha wagonjwa katika huduma za tiba na kutokuwa na ufuatiliaji stahiki wa watoto walio zaliwa na wazazi wenye VVU. Utafiti ulisisitiza uwepo wa taratibu rahisi na madhubuti za kuzuia maambukizi kutoka kwa mama kwenda kwa matoto kama Option B+, mbinu mpya ya kuunganisha huduma za afya ya uzazi na huduma za tiba ya VVU, na mrejesho wa mafunzo ya mara kwa mara yaliyotolewa kwa wafanyakazi wanaotoa huduma kwa

wajawazito wanaoishi na maambukizi ya VVU. Utafiti huu wa kwanza uliweka msingi wa uanzishwaji wa kliniki ijulikanayo kwa jina la One Stop Clinic, kitengo cha VVU cha Ifakara kinacho husika na mama na mtoto, kitengo kilichoundwa na kujumuishwa ndani ya huduma za afya ya uzazi na mtoto.

Utafiti wa pili na watatu ulichunguza matokeo ya huduma iliyo tolewa na One Stop Clinic kwa mama wajawazito wenye VVU, watoto na familia zao. Utafiti wa pili ulichunguza tabia/asili ya washiriki na matokeo ya matibabu na kudumu katika huduma kwa wanawake na watoto walio jiunga katika huduma ya VVU kabla(2008-2012) na baada (2013-2014), utekelezwaji wa One Stop Clinic ikiunganishwa na mabadiliko ya kuachana na matumizi ya makaratasi na kutumia elekitroniki na kutanuka wa huduma ya huduma ya upimaji na ushauri nasaha katika wodi za hospitali. Mbinu hii ilipelekea kuongezeko ya akina mama na watoto walio gundulika na VVU na kujiunga na huduma, ongezeko la watoto waliogundulika na UKIMWI, ongezeko la walio pata matibabu ya ARV, kupungua kwa wagonjwa wanaisitisha huduma na kupungua kwa maambukizi kutoka kwa mama kwenda kwa mtoto mpaka kufikia chini ya kiwango cha kutokomeza maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto. Hatimaye utafiti huu unatoa mrejesho wa mfumo wa matibabu ya VVU unaolenga familia nzima ambao unatekelezeka na unaweza kuenezwa kwa nchi zilizo kusini mwa jangwa la sahara.

Utafiti wa tatu ulichunguza tena kwa mara nyingine utekelezaji wa huduma za kuzuia maambukizi ya VVU katika eneo la Ifakara kwa mwaka 2014-2015. Ulitathmini matokeo ya maboresho yaliyotokana na utafiti wa kwanza sambamba na utekelezaji wa Option B+ ulioafikiwa na serikali ya Tanzania. Huduma ya kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto ulichunguzwa katika kliniki ya afya ya mama na mtoto, katika sehemu za kutolea huduma za VVU na wodi za wazazi na kulinganishwa na kabla ya kipindi cha utekelezaji wa Option B+. Utekelezwaji wa Option B+ katika kitengo cha One Stop Clinic umepelekea upimaji wa VVU kwa takribani watu wote wanao hudhuria katika kliniki ya mama na mtoto, kiasi kikubwa cha watu wenye VVU kujiunga na huduma na kuanza matibabu ya ARV, na maambukizi kutoka kwa mama kwenda kwa mtoto kupungua hadi asilimia 2.2%. Hatahivyo, upimaji wa VVU Kipindi cha mwishoni mwa ujauzito na katika wodi ya wazazi ilikua hafifu, na kubaki katika huduma za matibabu wakati wa kuanza ARV haukua wa kuridhisha. Utafiti huu hutoa taarifa juu ya mafanikio yaliyofikiwa kutokana na utekelezwaji wa Option B+ na kuainisha vikwazo vinavyo kabili utekelezaji wa mpango wa Option B+.

Utafiti wa nne ulionesha sifa za watoto wanaoishi na VVU Tanzania vijijini na usugu wa virusi vya Ukimwi kwa dawa za kupunguza makali ya UKIMWI. Usugu wa dawa hizi miongoni mwa

watoto wa Afrika umekuwa ukiripotiwa kwa uchache sana na hivyo utafiti huu umesaidia kutambua uwepo wa tatizo hili.

Matokeo ya utafiti huu wa nne ume vuta mawazo ya watu kuhusu uwepo wa tatizo unaohitaji mbinu madhubuti za utatuzi na ndilo lengo la utafiti wa tano. Watoto wote na vijana waliohudhuria One Stop Clinic Ifakara angalau kwa kipindi cha miezi kumi na mbili walishiriki katika utafiti huu. Kati ya watoto na vijana wakubwa 213, Asilimia 25.4% waligundulika kuwa kuzaliana kwa virusi vya Ukimwi katika miili yao haukusitishwa, 90% VVU walikua na usugu kwa dawa za kupunguza makali ya VVU, na 79% walikuwa na usugu kwa dawa zaidi ya moja. Kutokuwa na uzingataji mzuri wa dawa, jinsia ya kike na kutumia dawa yenye mchanganyiko wa NNRTI uliongeza uwezekano wa kuwa na usugu wa VVU. Kiasi kikubwa cha CD4 na umri mkubwa wakati wa kuanza ARV ilionekana kuwa ni kinga dhidi ya kupata usugu wa daw. Matokeo ya huu utafiti yanatoa taarifa muhimu kwa wataalamu wa afya na kwa watengeneza sera na kuibua maswali juu ya ubora wa programu matibabu ya ARV kwa watoto katika nchi za kusini mwa jangwa la Sahara. Matokeo ya utafiti huu hutangaza hitaji la kuimarisha mbinu za kuongeza uzingatiaji wa tiba, kutengenezwa kwa michanganyiko mipya ya dawa za watoto na uwepo wa vipimo vya idadi ya virusi mwilini.

Kwa kuhitimisha, utafiti huu umeonyesha kuwa mabadiliko ya kweli yanaweza kutekelezeka katika maeneo ya Tanzania vijijini. Programu ya One stop Clinic imepelekea ongezeko la upimaji wa VVU kwa wajawazito na kujiunga katika huduma za matibabu, Zaidi ya 90% ya hawa wajawazito walipata dawa kwa ajili ya kuzuia maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto, ambapo ni moja ya viwango vya juu vilivyowahi kuripotiwa kutoka sehemu za Afrika vijijini. Utekelezaji wa huduma hizi umepelekea maambukizi ya VVU kutoka kwa mama kwenda kwa mtoto kupungua hadi asilimia 2.2% tu. Mbinu za kutafuta watoto wenye VVU iliyotumiwa na One Stop Clinic ilifanikisha kugundua na kutibu watoto wenye maambukizi ya VVU ambao hapo mwanzo hawakugundulika. Zaidi ya yote, mbinu rafiki kwa watoto zimesaidia kutambua na kutathmini usugu wa VVU kwa dawa miongoni mwao, ambacho ni kikwazo kikubwa kwa matokeo mazuri ya matibabu kwa watoto. Kazi iliyofanyika ifakara imepelekea mrejesho huu wa mfumo unaotekelezeka na unaoweza kuenezwa ambao ukipelekwa pia katika nchi zingine za kusini mwa jangwa la Sahara unaweza kupelekea kutokomezwa kabisa kwa maambukizi mapya kwa watoto, kuwaweka wamama katika afya njema na kuziba nyufa katika matibabu ya VVU kwa watoto.

Acronyms and abbreviations

3TC Lamivudine

ABC Abacavir

AIDS Acquired Immune Deficiency Syndrome

ANC Antenatal Care Clinic

ART Antiretroviral Treatment

ARV Antiretroviral

ATV/r Ritonavir-boosted Atazanavir

AZT Zidovudine

BMI Body Mass Index

CDC Centers for Disease Control and Prevention

CDCI Chronic Diseases Clinic of Ifakara

CI Confidence Interval

CME Continuous Medical Education

D4T Stavudine

DRM Drug Resistance-associated Mutation

EFV Efavirenz

EID Early Infant Diagnosis of HIV

FTC Emtricitabine

HIV Human Immunodeficiency Virus

IQR Interquartile Range

KIULARCO Kilombero and Ulanga Antiretroviral Cohort

LPV/r Ritonavir-boosted Lopinavir

MTCT Mother-To-Child Transmission of HIV

NNRTI Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI Nucleoside/nucleotide Reverse Transcriptase Inhibitor

NVP Nevirapine

OR Odds ratio

PI Protease Inhibitor

PITC Provider-Initiated HIV Testing and Counselling

PLWHIV People Living With HIV

PMTCT Prevention of Mother-To-Child Transmission of HIV

RCHC Reproductive and Child Health Clinic

sdNVP single dose Nevirapine

SFRH St Francis Referral Hospital of Ifakara

SOP Standard Operating Procedure

SSA Sub-Saharan Africa

UNAIDS Joint United Nations Programme on HIV/AIDS

VCT Voluntary Counselling and Testing

VF Virological Failure

VL Viral Load

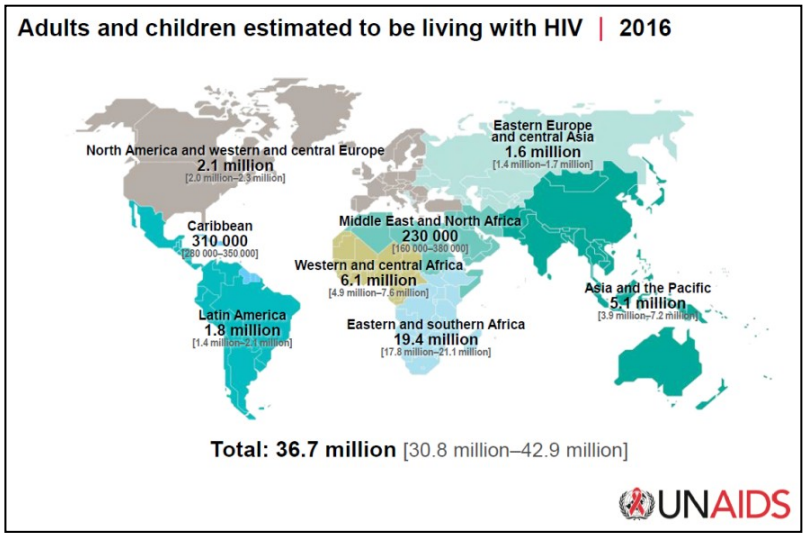
WHO World Health Organization

1. General Introduction

1.1. The Global burden of HIV/AIDS

HIV/AIDS is one of the worst pandemics in history. Since the first AIDS cases were reported in the United States in 1981 (Centers for Disease Control (CDC), 1981), all regions in the world have been affected, but sub-Saharan African countries bear a disproportionate share of the global burden.

Currently there are 36.7 million people living with HIV (PLWHIV) in the world. The most affected regions are Eastern and Southern Africa, where over 50% of PLWHIV reside. Much progress was made during the first decade of the 21st century, when the rate of new HIV infections in sub-Saharan Africa decreased by 50% between 2001 and 2011. During recent years this progress has slowed alarmingly, the number of new HIV infection in 2016 was 1.8 million, virtually the same that in 2010 (2.2 million) (UNAIDS, 2017).

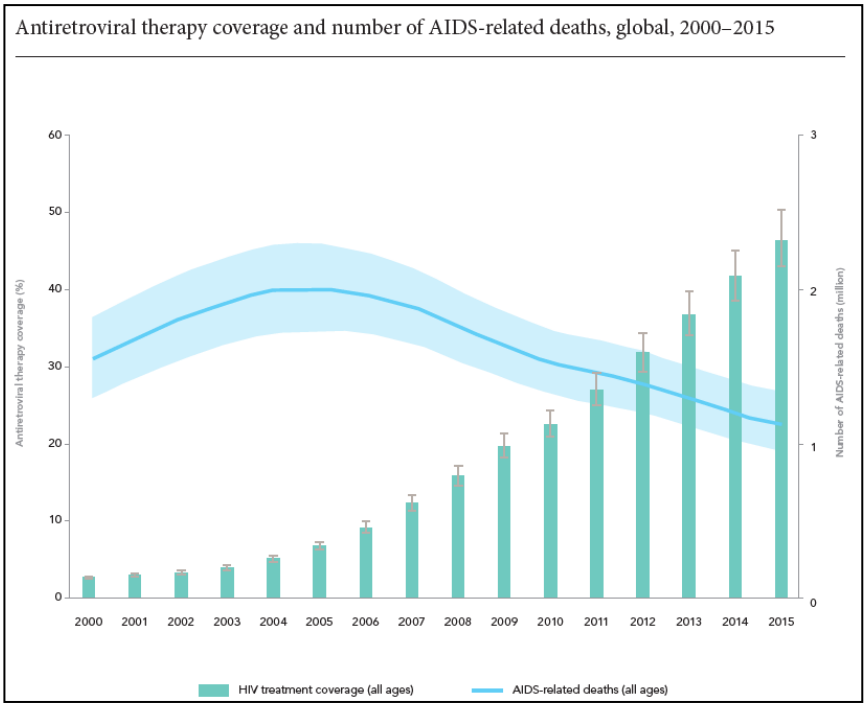


Source: UNAIDS estimates 2017

Before ART became available in sub-Saharan Africa, HIV infection resulted in premature death for most infected people (Morgan and Whitworth, 2001). Increased international funding, coupled with a dramatic reduction in antiretroviral prices about a decade ago (Badri *et al.*, 2006) has resulted in previously unimaginable access to ART (Reynolds and Quinn, 2010). ART roll-out in resource-limited settings constitutes one of the great achievements of global health in the last decade. Today, HIV-infected people on ART in sub-Saharan Africa can expect to have an overall life expectancy similar to that of HIV-negative individuals, and population-level

benefits include declining mortality rates in adults and children and increasing life expectancy (Bor et al., 2012, 2013; Tanser et al., 2013).

Through the years, evidence that earlier initiation of ART further delayed HIV disease progression emerged (When To Start Consortium *et al.*, 2009; Severe *et al.*, 2010; De Cock and El-Sadr, 2013; Le *et al.*, 2013). As a result, the WHO launched several revisions of its guidelines, and the recommended CD4 threshold for ART initiation progressively increased. Based on the latest evidence (INSIGHT START Study Group *et al.*, 2015; TEMPRANO ANRS 12136 Study Group *et al.*, 2015) the current WHO guidelines, published in September 2015, recommend initiating ART in all PLWHIV with any CD4 cell count. However, acknowledging the feasibility limitations of implementing these guidelines, a stepwise approach to implementation was recommended, determined by each country's capabilities. At the end of 2015, 17 million people were receiving HIV treatment worldwide, representing a global ART coverage of 46%. ART expansion has resulted in an annual AIDS-related deaths decrease since 2010 of 26% globally, and 38% in Southern and Eastern Africa (UNAIDS, 2016a).



Source: UNAIDS estimates 2016

Although the success in scaling up ART and stabilization of the epidemic must be acknowledged, challenges remain in Africa. Logistical factors prevent the full success of ART scale-up, key populations are sometimes neglected by country-specific guidelines, and the long survival and aging of the HIV-infected population complicates the management of HIV and its associated co-morbidities. Enhancement of local health systems is key to overcome these

challenges and be able to provide ongoing, sustainable, and comprehensive HIV care to the millions of people in need (Barker *et al.*, 2007; Mutevedzi and Newell, 2014).

1.2. Prevention of Mother-To-Child Transmission of HIV

The majority of paediatric HIV infections are acquired through mother-to-child transmission (MTCT), which can occur during pregnancy (in utero), during labour and delivery, or through the breast milk. Over 90% of paediatric HIV infections occur in sub-Saharan Africa, where 85% of pregnant women living with HIV reside (UNAIDS, 2014b). Without any preventive intervention, the MTCT rate among infants who breastfeed ranges from 25 to 40% (De Cock *et al.*, 2000).

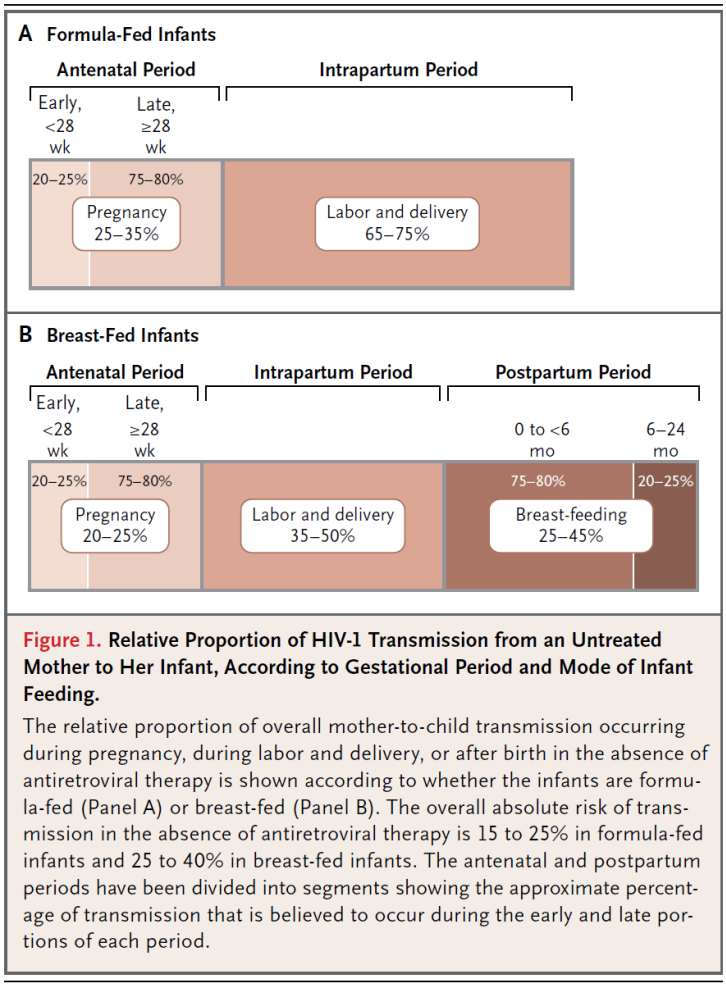


Figure 1. Relative Proportion of HIV-1 Transmission from an Untreated Mother to Her Infant, According to Gestational Period and Mode of Infant Feeding.

The relative proportion of overall mother-to-child transmission occurring during pregnancy, during labor and delivery, or after birth in the absence of antiretroviral therapy is shown according to whether the infants are formula-fed (Panel A) or breast-fed (Panel B). The overall absolute risk of transmission in the absence of antiretroviral therapy is 15 to 25% in formula-fed infants and 25 to 40% in breast-fed infants. The antenatal and postpartum periods have been divided into segments showing the approximate percentage of transmission that is believed to occur during the early and late portions of each period.

During the nineties, PMTCT was shown to be effective in well-resourced settings through the administration of antiretroviral prophylaxis to the mother during pregnancy, labour and delivery, and to the infant for the first six weeks of life (Connor *et al.*, 1994). Further evidence demonstrated that combined antiretroviral therapy given to the mother together with

elective caesarean section and avoidance of breastfeeding reduced MTCT to less than 1% (European Collaborative Study, 2005).

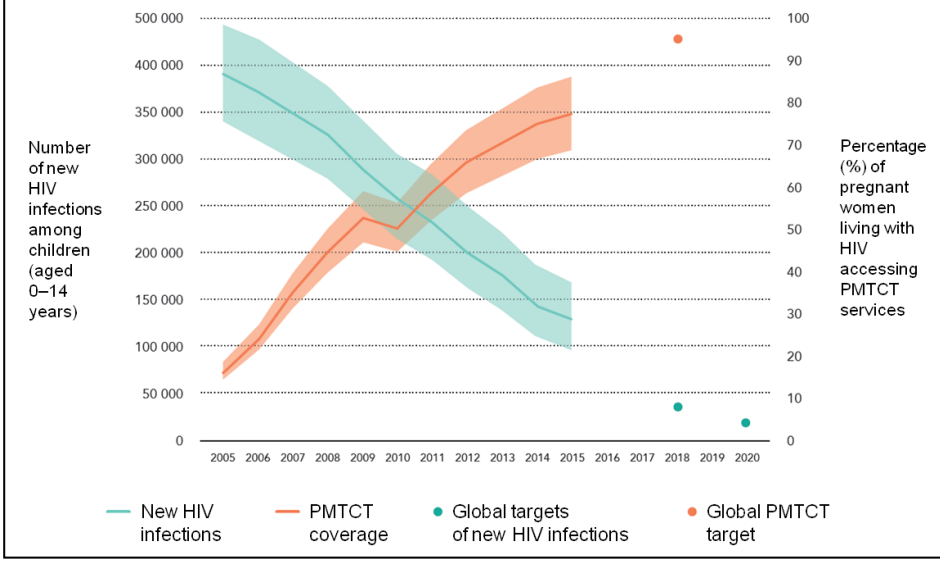
In most low income countries elective caesarean section and avoidance of breastfeeding are not safe or sustainable practices. The WHO issued the firsts PMTCT recommendations for resource limited settings in 2000. These guidelines have been revised, updated and simplified over the years to incorporate new evidence and to be aligned with the global commitment to universal treatment access and zero new infections among children.

In 2011, the UNAIDS presented "The Global Plan Towards the Elimination of New HIV Infections Among Children by 2015 and Keeping their Mothers Alive" (known as "Global Plan") (UNAIDS, 2011). The Global Plan recommends a four-pronged approach for PMTCT: 1) Prong 1 is prevention of HIV infections among women of childbearing age, 2) Prong 2 targets prevention of unintended pregnancies among HIV-infected women, 3) Prong 3 emphasizes the prevention of HIV transmission from infected women to their infants, and 4) Prong 4 includes provision of comprehensive treatment, care and support to mothers living with HIV and their families. To deliver this Prong 4 comprehensive PMTCT package the integration of HIV services for pregnant women, mothers, and children into the existing maternal and child health services is recommended.

Since 2012, the WHO recommends what is known as PMTCT "Option B+", meaning: lifelong ART for all pregnant and breastfeeding women regardless of their CD4 counts and clinical stage (WHO, 2012a, 2016). This option was first implemented in Malawi in 2011 (Schouten *et al.*, 2011), and given its ease of implementation and chances to increase PMTCT service coverage, improve maternal health and reduce transmission to serodiscordant sexual partners, was rapidly endorsed by the WHO. As of 2016, most sub-Saharan African countries had rolled out Option B+ (UNAIDS, 2015a).

According to UNAIDS, in 2014, in the Global Plan priority countries in Africa, 77% of HIV-infected pregnant women received antiretroviral drugs for PMTCT and the MTCT decreased from 28% in 2009 to 14% (UNAIDS, 2015a). This represents a 70% decline in the number of new HIV infections among children between 2000 and 2015 (UNAIDS, 2016b).

New HIV infections among children (aged 0–14 years) and percentage of pregnant women living with HIV receiving antiretroviral medicines (either prophylaxis or lifelong therapy) to prevent mother-to-child transmission

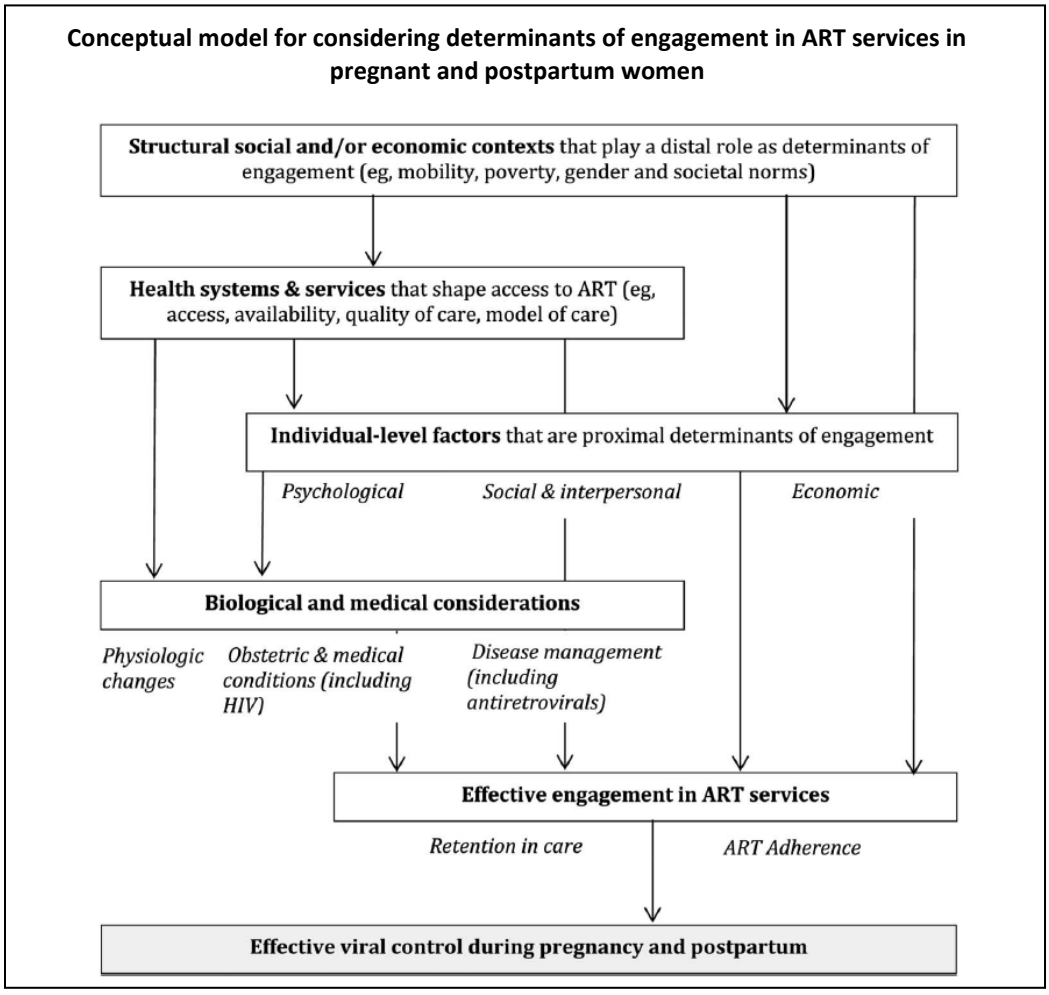


Source: UNAIDS estimates 2016

A crucial step of all PMTCT programs is Early Infant Diagnosis of HIV (EID), the HIV testing of infants to determine if they have acquired HIV. Given the rapid progression of HIV disease in untreated perinatally infected infants, timely diagnosis is critical. Maternal HIV antibodies are passively transferred to the baby across the placenta, and thus, assays that detect viral material (virological tests) are needed to diagnose HIV infection in infants. There are theoretical concerns about the use of HIV RNA virological testing in infants who have taken or are taking antiretroviral prophylaxis for PMTCT or in those who are breastfeeding from mothers on ART. While the performance of HIV RNA assays in specimens from infants is being assessed, the WHO recommends pro-viral HIV DNA PCR from whole blood or dried blood spots as the preferred method to detect HIV infection (WHO, 2010d). Despite the important progress on the numbers of pregnant women receiving antiretrovirals for PMTCT, testing of HIV-exposed infants is lagging behind. In 2014, only 49% of the estimated 1.2 million HIV-exposed infants received a virological HIV test within the firsts 2 months of life (UNAIDS, 2015a). This represents an important improvement from the reported 10% in 2009, but it is still alarmingly low, and undermines the opportunity to provide timely care and treatment to HIV-infected infants.

One of the main challenges of PMTCT programs derives from the length of the process. Prevention of vertical HIV infection starts with the first antenatal care visit and must be continued until the end of the breastfeeding period. In 2014, in the sub-Saharan Africa priority countries, the MTCT rate at 6 weeks of life was 5%, but rose to 14% at the end of the

breastfeeding period. Transmission during breastfeeding occurred mostly due to inadequate adherence and retention in care of HIV-infected mothers (van Lettow *et al.*, 2014; Kim *et al.*, 2015; Chan *et al.*, 2016; Haas *et al.*, 2016). PMTCT programs have been mostly focused on the antepartum component of prevention, and now emphasis on the follow-up and retention in care during the long breastfeeding period is needed. The integration of PMTCT and paediatric HIV care within the maternal and child health services is strongly recommended by WHO to improve the retention of HIV-affected mother-infants pairs (UNAIDS, 2011; WHO, 2014b). However, integration alone may not be sufficient. There is not a single risk factor that drives to the observed challenges in retention in care and adherence to ART during pregnancy and breastfeeding periods. In a recently published review, Myer and Philips propose a framework that recognizes the fundamental drivers of disengagement from HIV care of pregnant and postpartum women (Myer and Phillips, 2017).



Source: Beyond "Option B+": *Understanding Antiretroviral Therapy (ART) Adherence, retention in Care and Engagement in ART Services among Pregnant and Postpartum Women Initiating Therapy in Sub-Saharan Africa*. J Acquir Immune Defic Syndr. 2017 Jun 1; 75 Suppl 2: S115-S122

Intervention packages targeting this wide range of factors from different levels including health systems, individual psychological and social factors and biological aspects, need to be developed, implemented and tested.

1.2.1. HIV and Prevention of Mother-to-Child Transmission in Tanzania

Tanzania, in Eastern Africa, is one of the countries most affected by the HIV pandemic. In 2015, there were an estimated 1.4 million PLWHIV in the country, 91,000 being children (< 15 years). The HIV prevalence among adults (15-49 years) is 4.7% and there were 54,000 new HIV infections and 36,000 AIDS-related deaths during 2015 (UNAIDS, 2015b).

HIV care and treatment services are offered free of cost to all people in need through a network of clinics coordinated by the National AIDS Control Programme. The firsts clinics become operational in 2004 and currently there are more than 1,200 treatment centres located at referral, regional and district hospitals as well as primary health facilities (Tanzanian MoHSW, 2015).

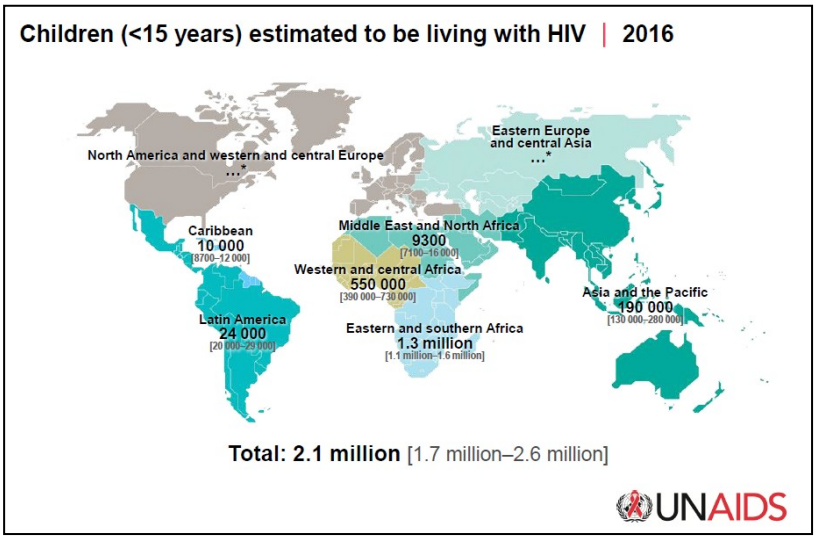
The PMTCT program in Tanzania started also in 2004, when the first guidelines were developed. The current guidelines were published in September 2013 and recommend Option B+ (Tanzanian MoHSW, 2013b). This option was progressively rolled out during 2013 and 2014 to all health facilities providing PMTCT care.

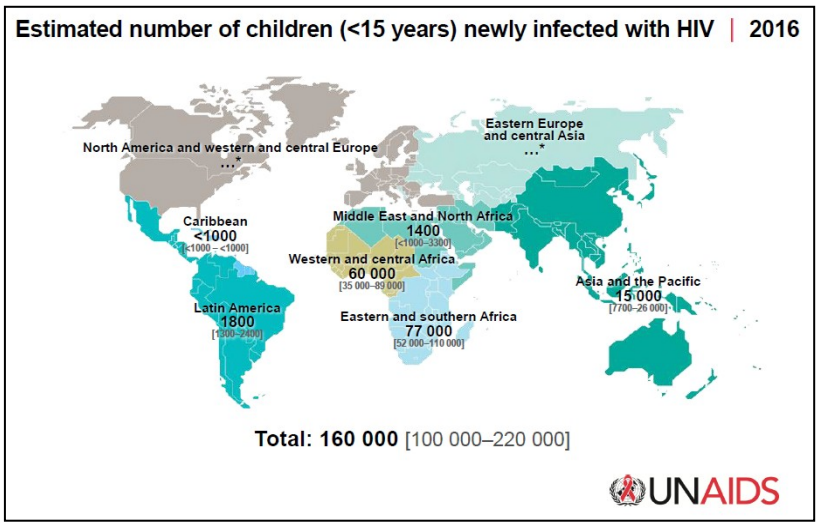
The Ministry of Health and Social Welfare recommends the provision of comprehensive treatment, care and support to mothers living with HIV and their families. To deliver this comprehensive PMTCT package is critical to link and integrate HIV health services within the reproductive and child health clinics. Such integration needs to be accompanied by a continuous training on HIV of the attending health workers in the antenatal and child health clinics, a strong supply chain of HIV tests, antiretroviral drugs and drugs for opportunistic infections, and a strategy to overcome shortages of staff. These requisites are frequently unmet and prevent an optimal implementation of Option B+.

In 2015, after Option B+ was already deployed to the whole country, 86% of pregnant women living with HIV received antiretrovirals for PMTCT (UNAIDS, 2015b). The reported MTCT rate in 2014 was 3% at 6 weeks of life and 9% at the end of breastfeeding, representing a 72% decline in the number of new paediatric HIV infections since 2009 (UNAIDS, 2015a). Similarly to other countries in sub-Saharan Africa, in 2015, only 43% of HIV-exposed infants received an EID test by the age of 2 months.

1.3. Children living with HIV

There are an estimated 2.1 million of children living with HIV in the world (UNAIDS, 2017). Over 90% of them reside in sub-Saharan Africa and MTCT accounts for over 90% of the infections (UNAIDS, 2013). Despite the laudable progress of PMTCT interventions implementation, 150,000 new infections occurred in 2015 (411 children infected every day), highlighting the number of women who do not access antenatal care or the necessary measures to prevent HIV transmission to their infants.

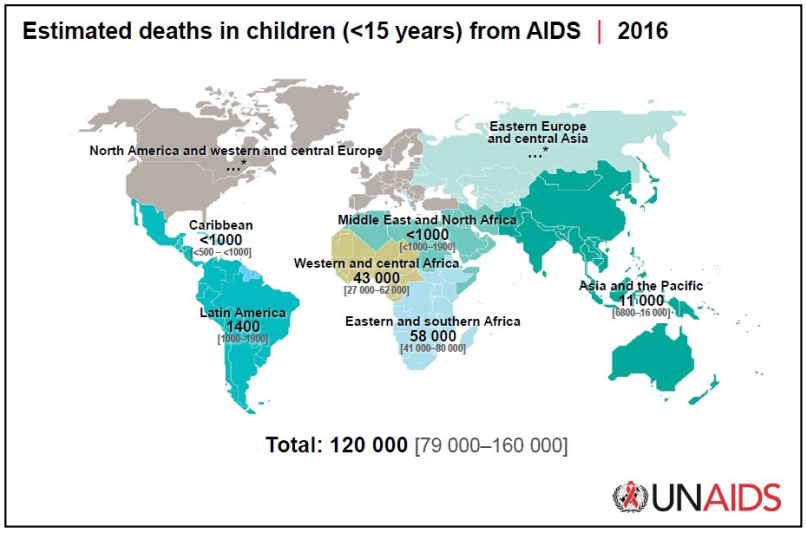




Source: UNAIDS estimates 2017

Progression of HIV diseases in children is more rapid than in adults. In sub-Saharan Africa, without treatment, 53% of infected children die before their second birthday and 75% before the age of three years (Newell *et al.*, 2004). This rapid disease progression and poor clinical outcomes make of vital importance the provision of EID for all HIV-exposed infants and early ART initiation for those who become infected. By the end of 2015, an estimated 5 million

children had died of AIDS-related causes since the start of the HIV epidemic. Only in 2016, 120,000 children died due to AIDS (over 320 children per day). Most of these deaths could have been avoided if HIV transmission had been averted in first place, but in cases in which infants were perinatally infected, early diagnosis and treatment would have reduced early infant mortality by 76% (Violari *et al.*, 2008).



Source: UNAIDS estimates 2017

Since 2010, earlier treatment for HIV-infected children has been progressively promoted. The WHO recommended, in 2010, to treat all children under the age of two years, irrespective of their clinical or immunological stage. In 2013, the age threshold was raised to five years (WHO, 2013) and, in 2015, treatment for all HIV-infected children was recommended (WHO, 2015).

Implementation of such recommendations and the scale-up of paediatric ART have encountered substantial challenges that have resulted in a slow progress and a concerning gap in treatment coverage. Of the 1.8 million children under 15 years of age living with HIV, only 49% accessed ART in 2015 (UNAIDS, 2016b). This is an improvement compared to 27% in 2013 and 10% in 2009, but still too many children are not accessing health-restoring treatment. Reasons for the slow progress of paediatric ART scale-up can be divided into: i) gaps identifying HIV-infected children, and ii) barriers to access ART.

Gaps identifying HIV-infected infants and children

Despite the remarkable progress of PMTCT programs in delivering antiretrovirals to HIV-infected pregnant women, the progress made with EID is less robust. As already mentioned in the previous section, in 2015, less than half of HIV-exposed infants got a virological test done within the firsts two months of life. A main reason for this low coverage is the low post-partum retention in care of mothers and the weak systems to track mothers and infants who

do not return for testing or results (Chatterjee *et al.*, 2011; Essajee *et al.*, 2017). Also, EID circuits are often affected by stock outs of testing kits or laboratory reagents, delays in sample collection, and long turn-around times of results (Nuwagaba-Biribonwoha *et al.*, 2010; Hsiao, Stinson and Myer, 2013).

Since PMTCT interventions include lifelong maternal ART the number of infections expected among infants born from mothers enrolled and retained in a PMTCT program is low. Thus, most HIV-infected infants and children are to be found outside the PMTCT-EID cascade. The WHO recommends opt-out screening for HIV in health care settings, meaning that an HIV test will be done routinely unless a patient explicitly refuses to take the test (WHO, 2007a). While opt-out HIV testing is generalized in most antenatal care settings, it is not as widespread in entry points where children at high risk of HIV seek for services: malnutrition units, tuberculosis clinics and in-patient wards (Ahmed *et al.*, 2013, 2016; Chamla *et al.*, 2013). Also, index testing is often forgotten. Testing children of adults living with HIV who are under care should be routine practice in all HIV clinics.

On the patients' side, it is important to consider the concerns of caregivers about paediatric HIV testing. Although most adults feel that HIV testing would benefit children, many fear the discrimination children may face in the community if they are infected (Buzdugan *et al.*, 2012).

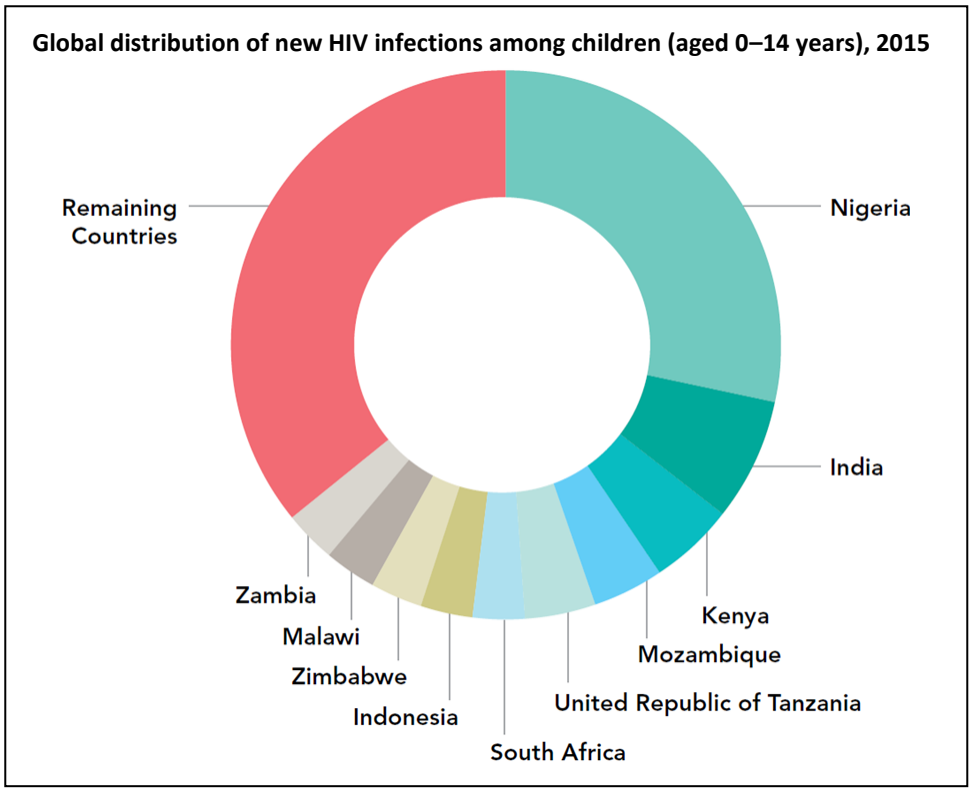
Barriers to access ART

Long waiting times, lack of integration and/or coordination with other child health services and difficulties with paediatric counselling and venipuncture are reasons caregivers cite for not accessing HIV services (Yeap *et al.*, 2010). The two key service delivery enablers to overcome these barriers are task shifting and the integration of paediatric ART into other health programs (Penazzato *et al.*, 2017). A systematic review published in 2014 suggested that nurse-managed paediatric HIV services had similar mortality and retention outcomes compared to physician-led clinics (Penazzato *et al.*, 2014). To ensure the provision of quality paediatric HIV care and safely overcome the perception that paediatric HIV/AIDS care and treatment are inherently complex, task shifting, or better, sharing between physicians and non-physicians, should be carefully planned and accompanied by robust training and ongoing supportive supervision and mentoring. As nurses become better able to diagnose, manage and treat HIV-infected children, paediatric HIV services can be decentralized and integrated into either the standard child health services or, with the adult ART services. Given the lifelong need of ART of all children living and growing with HIV, family–centred approaches within ART services may be a more sustainable approach.

A major barrier to start and retain children on treatment is the limited paediatric antiretroviral options. The availability of new formulations is likely to lead to further improvements in adherence and retention (DNDi, 2013). Along with the development of new formulations, ensuring that commodities are in place and minimizing shortages and stock-outs are critical to ensure the scale-up of paediatric ART and the improvement of clinical outcomes and survival of HIV-infected children.

1.3.1. Children living with HIV in Tanzania

Tanzania is the fifth country in the world with more children living with HIV. In 2015, it was estimated that 91,000 children were HIV-infected and 6,500 new paediatric infection occurred (UNAIDS, 2015b).



Source: UNAIDS estimates 2016

In 2014 only 29% of children (0-14 years) living with HIV in Tanzania received ART (UNAIDS, 2015a). However, the National AIDS Control Program recommended in June 2015 to treat all HIV-infected children regardless of their CD4 counts and percentage and clinical stage. Due to this change in the recommendations, many children that were already under HIV care but not receiving ART were started on medication. According to UNAIDS, by the end of 2015, ART coverage among children in Tanzania was 56% (UNAIDS, 2015b). Although this represents a

praiseworthy improvement, there is still a long way ahead to reach the targets of 90% of ART coverage (UNAIDS, 2014a, p. 90) and zero new paediatric infections.

1.3.2. Children and HIV drug resistance-associated mutations

The roll-out of ART in resource-limited countries resulted in a reduction of HIV-related morbidities and mortality, and increased the life expectancy of infected adults and children (Puthanakit *et al.*, 2007; Patel *et al.*, 2008). Long-term treatment success and virological suppression is harder to achieve in children compared to adults (van Rossum, Fraaij and de Groot, 2002). Children present poorer virological response, which is associated to high viral loads before treatment, the risk of sub-therapeutic drug concentrations caused by limited paediatric drug formulations, variable pharmacokinetics, and fast changes in bodyweight derived from growth (Abrams *et al.*, 1998; van Rossum, Fraaij and de Groot, 2002; Menson *et al.*, 2006). The restriction of reliable HIV test for infants and the limited laboratory capacity to monitor treatment efficacy in most resource-limited settings in combination with often already advanced levels of immunosuppression at treatment initiation further aggravate treatment outcomes among children (Sigaloff *et al.*, 2011; UNAIDS, 2013, p. 201). Together with a suboptimal adherence during childhood and adolescence, these factors promote the emergence of acquired HIV drug resistance mutations and reduce treatment options and the probability of virological suppression in this highly vulnerable population (Menson *et al.*, 2006; Bratholm *et al.*, 2010).

Until very recently, in resource-limited settings the initiation of ART has been mostly based upon CD4 counts and clinical criteria, thus leading to treatment initiation in children with advanced immunosuppression, which are prone to treatment failure (Bratholm *et al.*, 2010). In such settings, where paediatric formulations are scarce, it is common that children are prescribed halves of adult fixed-dose formulations, with the risk of an inaccurate dosage and subsequent sub-therapeutic drug levels. This, added to the low genetic barrier for some of the most often used drugs as nevirapine, causes an increased emergence of drug resistance mutations in children (Ellis *et al.*, 2007; Bratholm *et al.*, 2010).

Adherence to ART is a major factor for the emergence of drug resistance mutations and consequent treatment failure (Vreeman *et al.*, 2008; Bratholm *et al.*, 2010). Children's adherence depends on the caregiver, and the association between good adherence and a dutiful caregiver to achieve virological suppression is well established (Zoufaly *et al.*, 2013).

CD4 counts are commonly used for treatment monitoring, but have low sensitivity as indicator for treatment failure, detecting only one third of children failing to treatment. Delaying the detection of failing treatments causes the accumulation of resistance mutations and limits future second-line treatment options (Mutwa *et al.*, 2014). By performing regular virological monitoring patients would not be kept on a failing regimen and the development and accumulation of HIV drug resistance-associated mutations could be largely reduced. However, financial and technical limitations in many sub-Saharan African countries do not allow to perform such routine tests, thereby contributing to the emergence of HIV drug resistance mutations (Sigaloff *et al.*, 2011).

Virological success rates among children on ART vary widely between 40% and 81% (Sutcliffe *et al.*, 2008b; Ciaranello *et al.*, 2009). Drug resistance mutations are found in approximately 90% of all children failing on first-line ART, being as frequent as in adults presenting treatment failure. However, since virological failure rates are higher among children, this translates into an overall higher rate of emergence of acquired drug resistances in children (Sigaloff *et al.*, 2011). The available data on acquired HIV drug resistance from East African countries are highly heterogeneous, reaching rates between 14% and 58% (Bratholm *et al.*, 2010; Sigaloff *et al.*, 2011; Wamalwa *et al.*, 2013; Mutwa *et al.*, 2014).

2. Specific introduction to this thesis

2.1. Study 1

The first study presented in this thesis is an assessment of the PMTCT circuit in Ifakara during the period 2010 - 2011. The aim was to identify the circuit gaps to develop a strategy to enhance the uptake of the PMTCT recommendations and improve the outcomes of HIV-infected pregnant women and their offspring.

2.2. Study 2

The second study in this thesis evaluates the impact of a bundle of measures implemented to improve maternal and paediatric HIV care in Ifakara. The strategy designed was partially based in the assessment of Study 1. This study describes the baseline characteristics, clinical outcomes and retention in care of pregnant women and children enrolled in HIV care before (2008 – 2012) and after (2013 – 2014) the implementation of this bundle of measures.

2.3. Study 3

The third study of this thesis re-assesses the PMTCT cascade in Ifakara during the period 2014-2015. This study evaluates the impact of the measures taken after the first assessment presented in Study 1 combined with the simplification of PMTCT guidelines (Option B+) adopted by the Government of Tanzania after the WHO recommendation.

2.4. Study 4

The forth study focuses on the outcomes of children and adolescents enrolled in the clinic by describing a case series to raise awareness of a potential threat to paediatric HIV programs in Africa: the acquisition of drug resistance-associated mutations in HIV-infected children.

2.5. Study 5

The fifth study presented builds on the findings of Study 4. This study describes the clinical, immunological and virological outcomes associated with ART in the absence of regular virological monitoring among children and adolescents attending the HIV clinic in Ifakara and determines the risk factors associated with virological failure and drug resistance-associated mutations development.

3. Objectives and Research Aims

The general objective of this thesis is to improve our understanding of the challenges of PMTCT and paediatric HIV care in rural sub-Saharan Africa and to test a strategy with several interventions to improve the care of HIV-infected mothers, children and their families attending the Chronic Diseases Clinic of Ifakara.

3.1. Study 1

Uptake of Guidelines on Prevention of Mother-to-Child Transmission of HIV in Rural Tanzania - Time for change

Objectives:

- To describe the PMTCT cascade at the St Francis Referral Hospital in Ifakara.
- To describe the uptake of the PMTCT recommendations at the St Francis Referral Hospital in Ifakara.

Hypothesis:

- There are gaps in the PMTCT circuit that prevent the optimal implementation of the recommendations.

Specific aims:

- To identify the different departments and health workers involved in the PMTCT service delivery in Ifakara.
- To identify the gaps in the PMTCT cascade and their possible causes.
- To propose solutions adapted to the setting to bridge the identified gaps.

Relevance:

- This baseline assessment of the PMTCT cascade in Ifakara serves as the basis to design a strategy to improve the service delivery.

3.2. Study 2

An Integrated and Comprehensive Service Delivery Model to Improve Pediatric and Maternal HIV Care in Rural Africa

Objectives:

- To describe the One Stop Clinic of Ifakara: a package of measures to improve the quality of paediatric and maternal HIV care.
- To assess the impact of the One Stop Clinic on the diagnosis, linkage into care, treatment coverage and retention of HIV-infected pregnant women and children in Ifakara.

Hypothesis:

- The One Stop Clinic has contributed to improve the diagnosis, linkage into care, treatment coverage and retention of HIV-infected pregnant women and children in Ifakara.

Specific aims:

- To assess the number of new paediatric patients and pregnant women enrolled in care before and after the implementation of the One Stop Clinic.
- To describe and compare the baseline characteristics of the newly enrolled children and pregnant women before and after the implementation of the One Stop Clinic.
- To describe and compare the ART coverage before and after the implementation of the One Stop Clinic.
- To describe and compare the ascertainment of tuberculosis and malnutrition before and after the implementation of the One Stop Clinic.
- To describe and compare the retention in care 6 months post-enrolment before and after the implementation of the One Stop Clinic.
- To describe the cohort of HIV-exposed infants that is being followed up since the implementation of the One Stop Clinic.

Relevance:

- This manuscript is important to assess if the service delivery model of the One Stop Clinic improves the maternal and paediatric HIV care in a rural African setting as Ifakara.

3.3. Study 3

Prevention of Mother-To-Child Transmission Option B+ Cascade in Rural Tanzania: the One Stop Clinic Model

Objectives:

- To describe the uptake of PMTCT Option B+ guidelines during its first year of implementation in St Francis Referral Hospital in Ifakara and compare it with the previously described PMTCT cascade (Study 1).

Hypothesis:

- The simplification of the PMTCT recommendations with Option B+ combined with the integrated service delivery of the One Stop Clinic have resulted in a better uptake of the PMTCT guidelines.

Specific aims:

- To assess the uptake of PMTCT Option B+ guidelines in Ifakara.
- To compare this uptake with the previously described.

- To describe the current PMTCT circuit in St Francis Referral Hospital and identify the existing gaps and their possible causes.
- To assess the retention in care through pregnancy and post-delivery period, the pregnancy outcomes and the MTCT of HIV rate.

Relevance:

- This manuscript assesses the impact of the simplification of the PMTCT recommendations combined with the One Stop Clinic service delivery model on the PMTCT cascade.

3.4. Study 4

A Case Series of Acquired Drug Resistance-Associated Mutations in HIV-infected Children: an Emerging Public Health Concern in Rural Africa

Objectives:

- To raise awareness about a scarcely reported emerging public health concern in sub-Saharan Africa: the acquisition of drug resistance-associated mutation in HIV-infected children.

Hypothesis: not applicable

Specific aims:

- To present a well-characterised series of children from the One Stop Clinic of Ifakara with treatment failure due to the acquisition of drug-resistance mutations.

Relevance:

- This case series raises concerns about a scarcely reported threat for the success of treatment of children living with HIV.

3.5. Study 5

Development of HIV Drug Resistance and Therapeutic Failure in Children and Adolescents in Rural Tanzania – An Emerging Public Health Concern

Objectives:

- To objectively describe the clinical, immunological and virological outcomes associated with ART in the absence of regular virological monitoring among children and adolescents attending the One Stop Clinic of Ifakara and included in the KIULARCO cohort.

Hypothesis:

- Similarly to the unpublished data from the Tanzanian CDC, the virological failure rate among children in KIULARCO is around 40%.

- In approximately 90% of all children with virological failure, HIV drug resistance mutations can be identified.
- Virological failure and the presence of drug resistance-associated mutations are associated with (i) time since ART initiation, (ii) treatment adherence, (iii) under-dosage of drugs, and (iv) vital status of the parents.

Specific aims:

- To assess the prevalence of virological failure among the study patients.
- To assess the prevalence of HIV drug resistance-associated mutations among the study patients.
- To describe the clinical and immunological stage of the study patients at ART initiation and at the most recent visit.
- To compare the current drug resistance-associated mutations profiles with the available pre-ART sample to confirm the acquisition of resistances.
- To determine risk factors associated with virological failure and drug resistance-associated mutations development.

Relevance

- This study confirms the suspected high rates of virological failure among children/adolescents compared to adults and identifies the risk factors associated with it and HIV drug resistance mutations. By identifying these risk factors, measures to prevent virological failure and drug resistance mutations as well as to timely detect failing children can be developed.

4. Methods

4.1. Study site

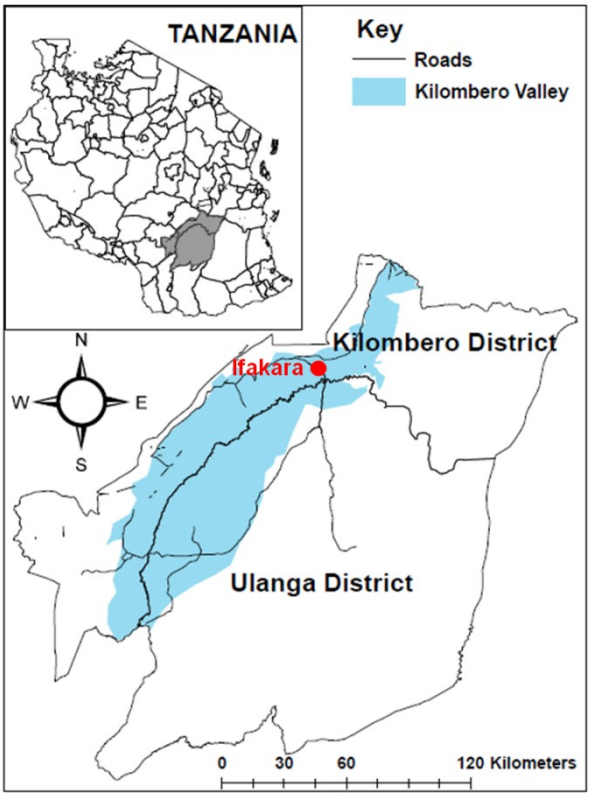
All the studies presented in this thesis are based on work undertaken at the Chronic Diseases Clinic of Ifakara. The clinic, located at the Kilombero district, southern Tanzania, was the first rural HIV care and treatment clinic accredited to provide HIV services in the country. It is part of the Saint Francis Referral Hospital and works in cooperation with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute and the Department of Infectious Diseases and Hospital Epidemiology of the University Hospitals of Basel and Bern, Switzerland. All patients diagnosed with HIV within the hospital or diagnosed at a peripheral health centre and transferred for further treatment are referred to the clinic to receive HIV care and treatment according to the National AIDS Control Program.

Since 2004, all HIV-infected patients attending the clinic are invited to participate in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO). KIULARCO was originally developed for monitoring and evaluating ART roll-out. The current objectives of the KIULARCO cohort are to: (i) provide patient and cohort-level information on the outcomes of HIV treatment; (ii) provide cohort-level information on opportunistic infections and non-AIDS co-morbidities; (iii) evaluate aspects of HIV care and treatment that have national or international policy relevance; and (iv) provide a platform for studies on improving HIV care and treatment, including clinical trials (Letang *et al.*, 2017; Vanobberghen *et al.*, 2017).

The Ifakara Health Institute institutional review board and the National Health Research Ethics Review Committee of the National Institute for Medical Research of Tanzania provided ethical approval for KIULARCO, including for sample collection, cryopreservation and analysis of collected data. Written informed consent is sought from all participants at registration at the Chronic Diseases Clinic of Ifakara; for children and adolescents younger than 18 years, informed consent is sought from caregivers.

The rural districts of Kilombero and Ulanga, in the Morogoro region of southern Tanzania, have a population of approximately 700,000 people (Tanzanian NBS, 2012) and an estimated 40,000 PLWHIV. The St Francis Referral Hospital is the largest health facility in the Kilombero district, is located in its main town, Ifakara, and serves as the referral hospital for the lower level hospital, health centers and dispensaries.

Since 2004, almost 9,000 PLWHIV have been enrolled in KIULARCO, and 6,858 (76%) have received ART (Letang *et al.*, 2017).



Kilombero and Ulanga districts in Tanzania

4.2. Study 1

Uptake of Guidelines on Prevention of Mother-to-Child Transmission of HIV in Rural Tanzania - Time for change

Research question: What was the uptake of PMTCT recommendations in Ifakara during 2010 - 2011?

Study population: Pregnant women attended in the antenatal care clinic, the HIV clinic or the labour ward in St Francis Referral Hospital during 2010 - 2011.

Study design: Situation analysis. Cross-sectional study retrieving data from the different stations where pregnant women receive health services at St Francis Referral Hospital.

Measurements: Absolute number of pregnant women attending the different stations and proportions of HIV testing, access to antiretroviral drugs, adherence to the PMTCT protocols, etc.

Statistical analysis plan: Purely descriptive study.

Sample size calculation: not applicable.

4.3. Study 2

An Integrated and Comprehensive Service Delivery Model to Improve Pediatric and Maternal HIV Care in Rural Africa

Research question: What has been the impact of the package of measures implemented in Ifakara to improve maternal and paediatric HIV care?

Study population: HIV-infected pregnant women and children, and HIV-exposed infants enrolled in the KIULARCO from January 1st, 2008 to December 31st, 2014.

Study design: Prospective cohort study comparing the periods before (2008 - 2012) and after (2013 - 2014) the implementation of the package of measures.

Measurements: Number of new paediatric patients and pregnant women enrolled in KIULARCO every year, baseline characteristics, incidence of tuberculosis and malnutrition, ART coverage and retention in care. Number of HIV-exposed infants enrolled, clinical characteristics and MTCT rate.

Statistical analysis plan: Baseline characteristics are summarized and reported with appropriate statistical measures. Comparisons between both periods are made using Chi-square and Wilcoxon rank sum test for categorical and continuous variables respectively.

Sample size calculation: not applicable.

4.4. Study 3

Prevention of Mother-To-Child Transmission Option B+ Cascade in Rural Tanzania: the One Stop Clinic Model

Research question: What was the uptake of PMTCT Option B+ recommendations in Ifakara during its first year of implementation (4/2014 - 3/2015)? How does it compare to the previously described (Study 1)?

Study population: Pregnant women attended in the antenatal care clinic, the Chronic Diseases Clinic of Ifakara (and enrolled in KIULARCO) or the labour ward in St Francis referral Hospital during the study period. HIV-exposed infants enrolled in the HIV clinic during the study period.

Study design: Prospective cohort study.

Measurements: Antental care clinic: number of women attended, tested for HIV, HIV prevalence. KIULARCO: number of newly-enrolled pregnant women, number of women

becoming pregnant during follow-up, clinical characteristics, pregnancy outcomes. Labour ward: number of women attended, tested for HIV, HIV prevalence. HIV-exposed infants: number of newly-enrolled infants, clinical characteristics, MTCT rate.

Statistical analysis plan: Absolute number of pregnant women attending the different stations and proportions of HIV testing and HIV positivity (prevalence). Clinical characteristics from KIULARCO database are summarized and reported with appropriate statistical measures. Comparisons between the two periods are made using Chi-square or Fisher exact test and Wilcoxon rank sum test for categorical and continuous variables respectively.

Sample size calculation: not applicable.

4.5. Study 4

A Case Series of Acquired Drug Resistance-Associated Mutations in HIV-infected Children: an Emerging Public Health Concern in Rural Africa

Research question: not applicable. This manuscript is a description of a worrisome observation from the One Stop Clinic.

Study population: Children detected throughout routine clinical care to present virological falirue.

Study design: Case series: observational descriptive study**.**

Measurements/exams: not applicable

Statistical analysis plan: not applicable

Sample size calculation: not applicable

4.6. Study 5

Development of HIV Drug Resistance and Therapeutic Failure in Children and Adolescents in Rural Tanzania – An Emerging Public Health Concern

Research question: What is the prevalence and determinants of virological failure and HIV drug resistance-associated mutations among children and adolescents attending the One Stop Clinic of Ifakara and included in the KIULARCO cohort?

Study population: HIV-infected children aged < 19 years, included in KIULARCO, who have been on ART for > 12 months.

Study design: Longitudinal study assessing virological failure and HIV drug resistance mutations among children and analysing prospectively collected data.

Measurements/exams: Prevalence and predictors of virological failure and HIV drug resistance mutations.

Statistical analysis plan: For data description, the numeric variables are displayed with medians and IQRs whereas the categorical variables are presented in proportions. Associations between considered variables and virological failure and HIV drug resistance mutations are assessed using multivariate logistic regression models.

Sample size calculation: Sample size was calculated under the expectation that the prevalence of virological failure in our setting was 40% (similar to the unpublished Tanzanian CDC data (Ward, J et al., 2014)). This prevalence is between the previously reported in Tanzania (58% (Bratholm *et al.*, 2010)) and the reported for resource-poor settings in a systematic review (24% (Sigaloff *et al.*, 2011)).

5. *Study 1*: Uptake of Guidelines on Prevention of Mother-to-Child Transmission of HIV in Rural Tanzania - Time for change

Swiss Med Wkly. 2013 Mar 14;143:w13775

Uptake of Guidelines on Prevention of Mother-to-Child Transmission of HIV in Rural Tanzania - Time for change.

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Summary

Guidelines on prevention of mother-to-child transmission (PMTCT) of HIV are inconsistently implemented in low-income countries. Strategies are needed to improve the uptake of these guidelines to prevent avoidable new HIV infections of infants. In 2010 the World Health Organization presented its new PMTCT guidelines, offering two options of short courses of antiretroviral prophylaxis: Option A and Option B. Option A consists of antenatal prophylaxis with zidovudine followed by intrapartum and postpartum prophylaxes with single dose nevirapine and zidovudine plus lamivudine, Option B recommends triple antiretroviral prophylaxis until finishing breastfeeding. Tanzania has adopted Option A, and it is currently implementing it. A new option termed Option B+ has emerged recently, which recommends providing lifelong antiretroviral treatment to all HIV-positive pregnant women.

In this article, we discuss the likely impact of this last PMTCT strategy in rural Africa with an example of an observational cross-sectional analysis in a rural referral hospital in Tanzania aiming to assess the uptake of PMTCT recommendations. Gaps were identified at all steps of the PMTCT pathway.

An effective uptake of PMTCT guidelines has shown to be extremely challenging in this setting. The continuously changing recommendations on PMTCT stress the need for a much simpler and effective approach. We argue in favor of implementing Option B+ in Tanzania. Financial challenges need to be faced, but Option B+ would help to overcome many barriers that prevent guidelines to be implemented in order to increase coverage and ultimately achieve the goal of "virtual elimination" of mother-to-child transmission in sub-Saharan Africa.

Different options for preventing mother-to-child transmission of HIV

More than two million children under the age of 15 are infected with Human Immunodeficiency Virus (HIV) worldwide, most of them in sub-Saharan Africa (UNAIDS, 2010b). Mother-to-child transmission of HIV accounts for over 90% of these cases (UNAIDS, 2010b), ranging the risk of transmission from 25 to 48% in resource-limited settings (De Cock *et al.*, 2000).

Prevention of mother-to-child transmission (PMTCT) was shown to be effective in well-resourced settings through the administration of antiretroviral prophylaxis to the mother during pregnancy, labour and delivery, and to the infant for the first six weeks of life (Connor et al. 1994). Further evidence demonstrated that combined antiretroviral therapy (cART) given to the mother together with elective cesarean section and avoidance of breastfeeding reduced mother-to-child transmission to less than 1% (European Collaborative Study 2005).

Consequently, guidelines were developed for high and in low income countries (WHO, 2010a; EACS, 2011).

The World Health Organization (WHO) issued the first recommendations on PMTCT in resource-limited settings in 2000. These recommendations were revised in 2004 with the adoption of simplified and standardized regimens. In 2006 and later in 2010 (WHO, 2010a), the guidelines were updated to incorporate new evidence and to be aligned with the global commitment to universal access.

Guidelines for resource-limited settings are difficult to implement, partly due to the fragility of health systems in these countries. Lack of infrastructure and constraints on human and financial resources contribute to a poor uptake of evidence based interventions and, ultimately, to the impairment of clinical practice in these settings. Dissemination of changes in the guidelines is suboptimal and often takes time to be rooted among health workers and to change practices in rural sub-Saharan Africa (Nkonki et al. 2007). According to WHO, only 53% of pregnant women worldwide received any antiretroviral for PMTCT in 2009 (WHO, 2010c), with substantial differences across countries in sub-Saharan Africa (i.e. 1% in the Democratic Republic of the Congo versus 52% in Mozambique) (UNAIDS, 2010a). Importantly, having effective antiretroviral regimens and wide coverage is insufficient, being effective delivery programmes equally important (Barker, Mphatswe and Rollins, 2011; Ciaranello *et al.*, 2012). Simplification of the existing programmes is urgently needed in order to bridge the gaps observed with current recommendations.

In 2010 the WHO presented its new guidelines on PMTCT, recommending two PMTCT options: Option A and Option B. These two options include both treatment and prophylaxis components. In both options CD4 cell count is necessary to decide the eligibility of HIV-infected pregnant women for lifelong cART. For all women who have CD4 cell counts ≤350 cell/mm^3 initiation of lifelong cART is recommended. For those women non eligible for lifelong cART while Option A recommends antenatal prophylaxis with zidovudine followed by intrapartum and postpartum prophylaxes with single dose nevirapine and zidovudine plus lamivudine, Option B recommends triple antiretroviral prophylaxis until after finishing breastfeeding. Recently a new option has emerged, termed Option B+ (WHO, 2012a). This option adapts a single, universal regimen both to treat HIV-infected pregnant women and to prevent mother-to-child transmission. In Option B+ all HIV-positive pregnant women are provided lifelong cART, regardless of the CD4 cell count. Option A might not bridge the implementation difficulties due to the changes in drugs delivered across the care continuum

(antenatal, delivery and postpartum). Option B and B+, while simplifying the drugs prescription, have a short-term drug cost greater than Option A. However, when having into account maternal and infant life expectancy and lifetime healthcare costs, option B is more effective and less expensive than Option A. Option B+ offers clinical benefits and economic value comparable with other widely used HIV interventions (Ciaranello *et al.*, 2013). The three options are summarized in Table 1.

Table 1. Options for preventing Mother-to-Child Transmission of HIV: Option A, Option B and Option B+

	Woman receives		**Infant receives**
	Treatment (CD4 counts ≤ 350 cells/mm^3)	**Prophylaxis** (CD4 counts > 350 cells/mm^3)	
Option A	Triple ARVs starting as soon as diagnosed and continued for life	*Antepartum:* AZT starting as early as 14 weeks gestation *Intrapartum:* at onset of labour, sdNVP and first dose of AZT/3TC *Postpartum:* daily AZT/3TC through 7 days postpartum	*Mother received prophylaxis*: daily NVP from birth until 1 week after cessation of all breastfeeding; if not breastfeeding through age 4–6 weeks *Mother is on treatment:* daily NVP through age 4–6 weeks
Option B	Triple ARVs starting as soon as diagnosed and continued for life	Triple ARVs starting as soon as 14 weeks gestation and continued intrapartum and through childbirth if not breastfeeding or until 1 week after cessation of all breastfeeding	*Irrespective of mode of infant feeding:* daily NVP or AZT from birth through age 4-6 weeks
Option B+	Same for treatment and prophylaxis Regardless of CD4 count, triple ARVs starting as soon as diagnosed and continued for life		*Irrespective of mode of infant feeding:* daily NVP or AZT from birth through age 4-6 weeks

Note: "Triple ARVs" refers to the use of one of the recommended 3-drug fully suppressive treatment options. HIV: Human Immunodeficiency Virus; AZT: Zidovudine; sd NVP: single dose Nevirapine; 3TC: Lamivudine; NVP: Nevirapine.

Several countries in sub-Saharan Africa are currently considering modifying their PMTCT guidelines. Such a consideration should be decided on the basis of their implementation experience and a previous assessment on how they can better integrate, simplify and optimize the PMTCT programme in the existing HIV/AIDS care and treatment platform.

The Tanzanian national guidelines on PMTCT were developed in 2004 and revised in 2007 (Tanzanian MoHSW, 2007b). New guidelines recommending WHO option A were developed in June 2012 and plans are being devised to implement them nationwide (Tanzanian MoHSW, 2012).

A cross-sectional survey in a referral hospital in Tanzania assessing the uptake of the current PMTCT recommendations – an example for problems encountered in rural sub-Saharan Africa

In this review we illustrate encountered problems of PMTCT with findings of a recently performed survey conducted in March 2012 in a referral hospital in Tanzania. Data on PMTCT services delivered between January 2010 and December 2011 at St. Francis referral hospital in Ifakara, Kilombero district, Morogoro region, Southern Tanzania, were collected. Table 2 summarizes the antiretroviral regimens recommended by the Tanzanian national guidelines on PMTCT at different steps. In this article, we will use this setting as a case-study to discuss the limitations on the implementation of guidelines, identify the gaps to be bridged, and envisage potential solutions, including the adoption of newer PMTCT strategies.

Table 2. Recommended combination antiretroviral prophylaxis regimens to prevent mother-to-child transmission of HIV according to the 2007 Tanzanian national guidelines for the Prevention of Mother-To-Child Transmission of HIV

REGIMEN	ANTENATAL	INTRAPARTUM	POSTPARTUM	POSTNATAL
Recommended	AZT 300 mg twice a day starting at 28 weeks of gestation or as soon as possible thereafter	AZT at 300 mg at onset of labour and every 3 hours until delivery sdNVP 200mg at onset of labour 3TC 150 mg at onset of labour and every 12 hours until delivery	AZT 300 mg twice daily for 7 days 3TC 150 mg twice a day for 7 days	sdNVP 2mg/kg immediately after birth AZT 4mg/kg twice a day for 7 days
Recommended if mother presents during labour		AZT at 300 mg at onset of labour and every 3 hours until delivery sdNVP 200mg at onset of labour 3TC 150 mg at onset of labour and every 12 hours until delivery	AZT 300 mg twice daily for 7 days 3TC 150 mg twice a day for 7 days	sdNVP 2mg/kg immediately after birth AZT 4mg/kg twice a day for 28 days
Recommended if mother tests HIV positive immediately after delivery			Refer to Care and Treatment Clinic, do not give any antiretroviral	sdNVP 2mg/kg immediately after birth AZT 4mg/kg twice a day for 28 days
Recommended if mother was on ART before pregnancy	Continue ART prescribed before pregnancy. In the first trimester replace EFV with NVP			AZT 4mg/kg twice a day for 7 days

AZT: Zidovudine; sdNVP: single dose Nevirapine; 3TC: Lamivudine; ART: Antiretroviral Therapy; EFV: Efavirenz; NVP: Nevirapine.
Source: Tanzanian national guidelines for the Prevention of Mother-To-Child Transmission of HIV. The United Republic of Tanzania, Ministry of Health and Social Welfare, 2007 [14]

Setting at a rural referral hospital

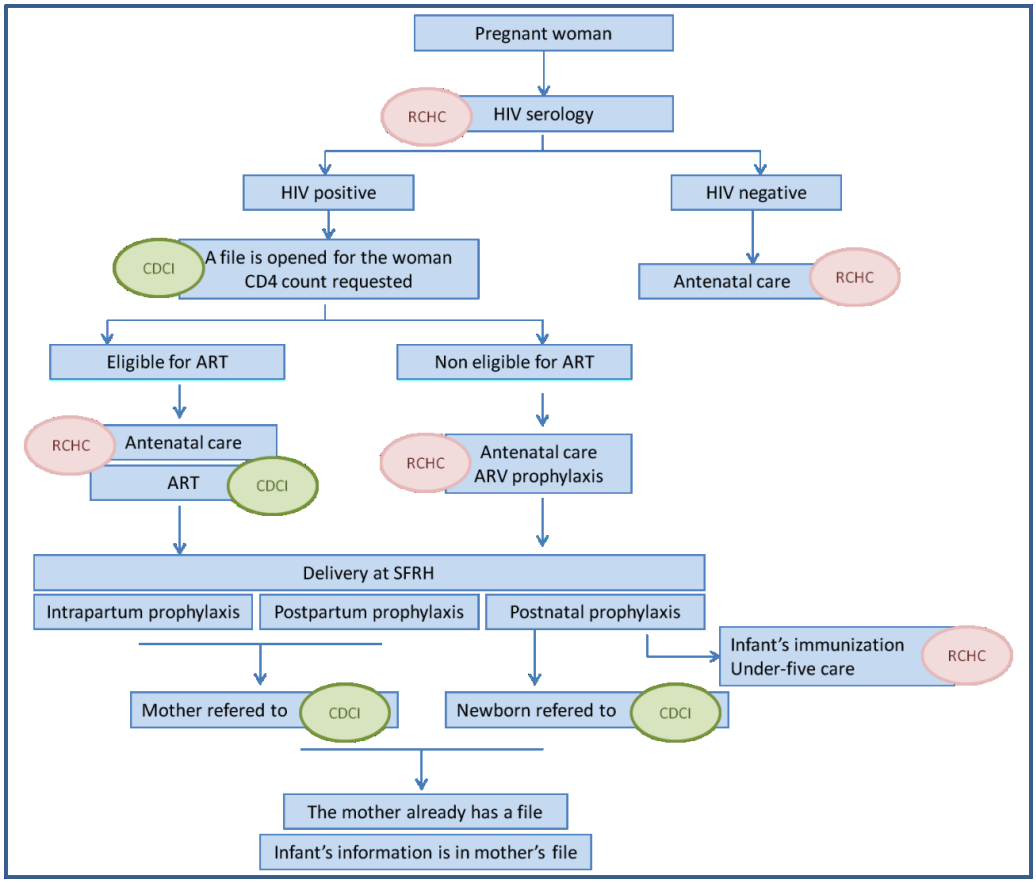
St. Francis referral hospital has 370 beds and is the most important health care facility in the Kilombero district. PMTCT services are provided between several departments in this hospital, including the Reproductive and Child Health Clinic; the Chronic Diseases Clinic of Ifakara; the Obstetrics department; and the Neonatology unit.

The Reproductive and Child Health Clinic provides antenatal care to all pregnant women as well as vaccinations and outpatient care for children under-five years old. Care and treatment for all HIV-positive patients is provided at St. Francis referral hospital according to the National AIDS Control Programme through the Chronic Diseases Clinic of Ifakara. The Chronic Diseases Clinic of Ifakara works in cooperation with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute and the Department of Infectious Diseases and Hospital Epidemiology of the University Hospital of Basel and Bern, Switzerland. All patients attending the Chronic Diseases Clinic of Ifakara since late 2004 are offered informed consent to be enrolled at the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) (Stoeckle *et al.*, 2006). This cohort comprises almost 6000 patients and is the largest peripheral HIV cohort in Tanzania.
The Obstetrics department attends approximately 5000 deliveries per year and works closely with the Neonatology unit.

PMTCT Circuit at St. Francis referral hospital and gaps identified

Figure 1 shows the theoretical PMTCT pathway at St. Francis referral hospital. The departments involved in this process are the following: Reproductive and Child Health Clinic, Chronic Diseases Clinic of Ifakara, Obstetrics, and Neonatology. The following key steps of the PMTCT pathway were identified: 1) HIV counseling and testing; 2) CD4 count measurement and eligibility for cART; 3) antenatal antiretroviral prophylaxis; 4) intrapartum and postpartum prophylaxis; 5) postnatal prophylaxis for the infant; 6) follow-up of HIV-positive mothers; and 7) follow-up of HIV-exposed infants.

Figure 1. Theoretical PMTCT of HIV care pathway at St. Francis Referral Hospital



RCHC: Reproductive and Child Health Clinic; HIV: Human Immunodeficiency Virus; PMTCT: Prevention of Mother-To-Child Transmission; CDCI: Chronic Disease Clinic of Ifakara; ART: Antiretroviral Therapy; ARV: antiretroviral; SFRH: St. Francis Referral Hospital.

According to WHO and Tanzanian national guidelines, all pregnant women attending the Reproductive and Child Health Clinic for antenatal care have to be offered HIV counseling and testing. Between January 2010 and December 2011, 4027 pregnant women attended the Reproductive and Child Health Clinic. Forty-four (1%) were already known to be HIV-infected. The remainder was offered HIV testing, with a rate of acceptance of 90.4% (3606/3983). Two hundred and seven of these 3606 women tested positive. Thus, the HIV prevalence among pregnant women attending antenatal services at St. Francis referral hospital during the study period was 6.9% (251/3647). Current Tanzanian guidelines recommend HIV seronegative women to be re-tested after three months in order to avoid falsely negative diagnoses during the "window period" after acute infection, but there was no information concerning the proportion of seronegative women who were re-tested. Information could only be extracted from randomly selected individual pregnancy cards, and none of the seronegative women assessed had a second test registered.

Those women who are newly diagnosed with HIV infection at St. Francis referral hospital must be referred to the Chronic Diseases Clinic of Ifakara. However, only 25.6% (53/207) of newly diagnosed women visited the clinic during 2010-2011 and were assessed for cART eligibility. Seventy-four percent (154/207) of HIV-infected women were not registered at the Chronic Diseases Clinic and thus cART eligibility criteria were not assessed. Consequently, all HIV-positive pregnant women with unknown CD4 count were prescribed antenatal antiretroviral prophylaxis in the Reproductive and Child Health Clinic regardless of the eligibility criteria for cART initiation. According to the labour ward PMTCT log book the antenatal prophylaxis prescribed to all HIV-positive women was monotherapy with nevirapine, which was not a recommended option (Table 2). This contradicts the information collected at the Reproductive and Child Health Clinic regarding antenatal prophylaxis prescribed during the same period, which was registered to be zidovudine.

All HIV-infected pregnant women are advised to deliver at St. Francis referral hospital to ensure optimal intrapartum and postpartum antiretroviral prophylaxis for the mother and postnatal prophylaxis for the infant. Between 20/03/2011 and 03/05/2011, 570 women delivered at the hospital. Almost 5% (28/570) and 80% (456/570) were known to be HIV-positive and HIV-negative respectively. The HIV serostatus was unknown for 14.4% (82/570) of women. Of those, 2.4% (2/82) were tested during labour with one testing HIV-positive. In total, 5.9% (29/486) of all women with a known HIV serostatus giving birth at St. Francis referral hospital during that period tested positive.

Concerning intrapartum prophylaxis, there was conflicting information between the register books and the interviews. The nurses interviewed did not appear to be aware of the latest recommendations on intrapartum prophylaxis, which include three drugs to be given at different intervals (Table 2). In contrast, 86% (25/29) of the HIV-positive women were registered to have been given correct prophylaxis with three drugs, and no information was registered for the remaining four women.

Regarding postpartum prophylaxis no information was recorded for any women. The interviews to staff members of the labour and the obstetric wards, suggest a lack of clarity with regards to the responsible service for its administration.

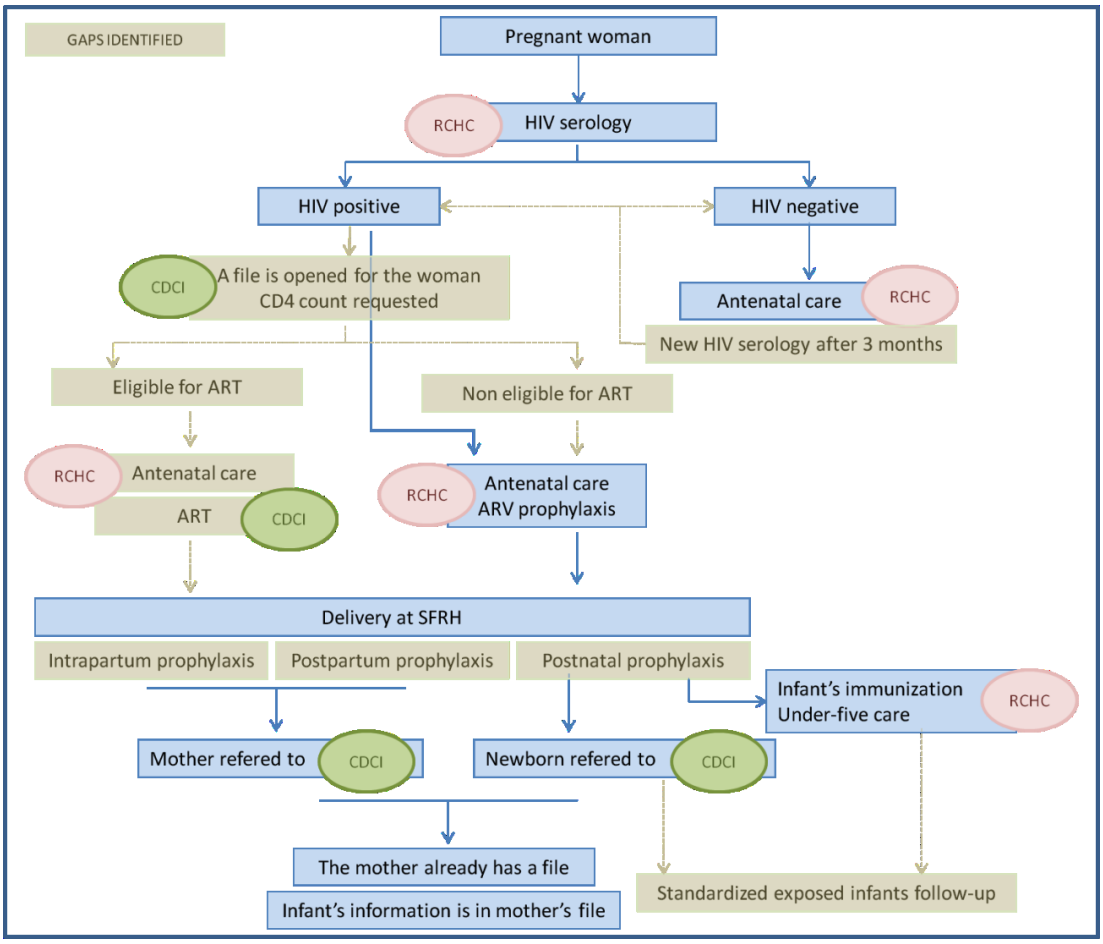
As postnatal prophylaxis, 83% (24/29) of the babies born from HIV-seropositive women were given single dose nevirapine, one (3.4%) received single dose nevirapine plus zidovudine for one week and no data was collected for the remaining four babies. This represents another

important gap in this pathway, since the postnatal prophylaxis recommended in the guidelines includes both single dose nevirapine plus zidovudine, very rarely administered at St. Francis referral hospital during the period assessed.

After delivery, mother and child should be referred to the Chronic Diseases Clinic of Ifakara, where the follow-up must be carried out. During 2010-2011 only one woman with an infant younger than two months was registered at the Chronic Diseases Clinic. There is no information with regards to the number of the HIV-exposed infants attending the clinic during the same period. There is neither active case finding nor a standardized follow-up for HIV-exposed infants in place. Consequently, the infant is visited together with the mother and information related to the exposed-non-infected child is not collected in any database.

Figure 2 illustrates the gaps identified in the PMTCT algorithm. For every gap identified at different steps of the PMTCT pathway the possible causes were assessed and potential solutions were suggested. Table 3 summarizes the most likely causes and the proposed solutions to close the specific gaps.

Figure 2. Main gaps identified at PMTCT of HIV care pathway at St. Francis Referral Hospital



The gaps are colored in brown. RCHC: Reproductive and Child Health Clinic; HIV: Human Immunodeficiency Virus; PMTCT: Prevention of Mother-To-Child Transmission; CDCI: Chronic Disease Clinic of Ifakara; ART: Antiretroviral Therapy; ARV: antiretroviral; SFRH: St. Francis Referral Hospital.

Table 3. PMTCT care pathway: observed gaps, possible causes, and potential solutions

STEP INVOLVED	OBSERVED GAP	POSSIBLE CAUSES	POTENTIAL SOLUTIONS
HIV testing at RCHC	Not all seronegative pregnant women are re-tested after 3 months of the 1st HIV negative serology	Unawareness about the "window period" after the primary HIV infection	CME. Refreshing seminars for all the staff involved in the antenatal care SOP
CDCI	After being diagnosed HIV-positive, most pregnant women do not attend the CDCI, so CD4 count is not performed and ART eligibility is not assessed	Pregnant women do not seem to feel comfortable attending the CDCI. According to the nurses' interviews, they may feel ashamed due to stigma.	Integrate the HIV-positive pregnant women care into the RCHC Option B+
Antenatal prophylaxis	There is a discordance between the ARV drugs prescribed in the RCHC and the ARV drugs registered in the labour ward books	Probable mistake when filling the books at the labour ward	CME. SOP Option B+
Delivery	There is not enough information to assume that intrapartum prophylaxis is correctly administered	Doses and frequency of the ARV are not easy to remember	CME. SOP. Poster with the intrapartum prophylaxis Identify a staff member responsible for checking the availability of ARV drugs in the labour ward Option B+
Post-Delivery	There is no data supporting that postpartum prophylaxis is being administered in the hospital	The staff does not appear to clearly know who is supposed to administer it (labour ward *vs*. obstetric ward) Lack of availability of ARV drugs in labour ward/obstetric ward Doses and frequency of the ARV are not easy to remember	Clearly assign responsibilities and duties SOP Identify a staff member responsible for checking the availability of ARV drugs in the labour ward/obstetric ward Poster with the postpartum prophylaxis. Counsel the mother about ARV prophylaxis. Option B+
Perinatal period	Postnatal prophylaxis is not always correctly administered after discharge	The staff does not seem to clearly know who is supposed to administer it (labour ward *vs*. obstetric ward *vs*. neonatal unit) Lack of availability of ARV syrups Doses and frequency are not easy to remember	Because the sooner the dose is given the greater the protective effect: labour ward should be the responsible Identify a staff member responsible for checking the availability of ARV syrups in the labour ward Poster with the postnatal prophylaxis. Counsel the mother about ARV prophylaxis for the baby SOP Option B+
Exposed infants follow up	There is not a standardized follow up for the exposed infants	The Guidelines do not specify about where the follow up should be done, what frequency, etc	Integrate the HIV-exposed infants follow up into the existing under-five health services. SOP Generate special files for the exposed infants. Attach these files to the mothers' CDCI files.

PMTCT: Prevention of Mother-To-Child Transmission; RCHC: Reproductive and Child Health Clinic; HIV: Human Immunodeficiency Virus; CME: Continuous Medical Education; SOP: Standard Operating Procedures; ART: Antiretroviral Therapy; ARV: Antiretroviral; AZT: Zidovudine; 3TC: Lamivudine; NVP: Nevirapine;

Propositions to increase the uptake of current and upcoming PMTCT guidelines - Time for change

The uptake of the guidelines in sub-Saharan Africa is known to be poor. The aim of this case presentation was to illustrate the quality of PMTCT provision at a rural Tanzanian referral hospital by providing a snapshot of the reality in the field and to identify existing gaps and potential ways to bridge them at an operational level. Our findings should help to develop new potential strategies to be tested in a prospective fashion with the aim of improving the uptake of PMTCT guidelines in rural Tanzania and other similar settings in sub-Saharan Africa. Moreover, simpler and more effective approaches need to be taken into account.

In St. Francis referral hospital, all the services and most resources needed for a proper functioning of the Tanzanian national PMTCT program were in place. However, several gaps were observed that prevented the current Tanzanian recommendations to be optimally implemented. This is worrisome, since it may ultimately result in an increasing rate of HIV infection among infants.

The most important gaps identified in the PMTCT care pathway were: a) no re-testing of seronegative pregnant women in late pregnancy; b) suboptimal follow-up of HIV-infected pregnant women, including irregular assessment of cART eligibility; c) inconsistencies in the prescription of antiretroviral prophylaxes; and d) lack of a standardized follow-up of HIV-exposed infants.

As mentioned, the Tanzanian Government has adopted Option A for the new PMTCT Guidelines. Lower short-term costs might justify the adoption of this strategy in Tanzania. However, the drug prices have been reduced since 2010, when the WHO recommendations were published, and they are expected to further decrease, narrowing the cost difference between Option A and B. Concerns are raised about the lack of simplification when transitioning from the former recommendations to Option A. Even if the higher CD4 count threshold for cART eligibility, the earlier initiation of antenatal prophylaxis and the longer postnatal prophylaxis for breastfeeding infants are definitely welcome modifications, the complexity of the circuit remain high and might prevent the correct implementation in the real world, as shown in the case presented. Simpler strategies are needed to increase the coverage and the efficacy of these programs. Better infrastructure and human resources must accompany these new strategies to avoid logistic problems as drug stock-outs (Pasquet *et al.*, 2010) and overburdened health staff.

Malawi adopted in 2011 Option B+, for its ease of implementation and potential prevention benefit (Schouten *et al.*, 2011). Likewise, other countries, such as Uganda and Swaziland, are also considering to move to Option B+. Using the case presented as a starting point, we are now going to discuss every gap observed and envisage what would be the situation if Option B+ was the adopted PMTCT recommendation in Tanzania.

HIV testing was conducted once and not repeated during the third trimester among those women who were initially seronegative. In a South African study (Moses *et al.*, 2008), 3% of initially seronegative pregnant women were detected to be HIV-infected in late pregnancy, showing the relevance of re-testing during the third trimester. Acute HIV infection during pregnancy is associated with high rates of vertical transmission due to the extremely high HIV RNA levels achieved during this phase of the infection, which is associated with higher probabilities of vertical transmission (Garcia *et al.*, 1999). Option A, B or B+ would not bridge this gap, since the awareness of the importance of re-testing has to be spread through educational efforts.

Regarding the assessment of the cART eligibility, the current Tanzanian guidelines during the time the analysis was performed recommended offering cART to all pregnant women with CD4 counts ≤200 cells/mm^3 or ≤350 cells/mm^3 in case of WHO stage 3 or 4. However, our analysis shows that after being diagnosed HIV-positive, most pregnant women were not assessed for cART eligibility. Instead, most were offered antiretroviral prophylaxis regardless of CD4 cell count. We have to take into account the possibility of some women attending another clinic than the Chronic Disease Clinic of Ifakara, but we do not consider this is a common or representative situation. With Option B+, all HIV-positive pregnant women would receive cART, avoiding suboptimal antiretroviral prophylaxis to women in need of cART for their own health.

In relation to the antenatal prophylaxis prescribed, inconsistencies in the antiretroviral regimens administered and the records in different services were observed. Concerning intrapartum prophylaxis, the Tanzanian PMTCT guidelines recommended using a combination of zidovudine, lamivudine, and nevirapine. Our findings show again irregularities in this crucial step. According to a study conducted in Malawi, skilled attendance at birth appeared to be an important determinant of correct intrapartum prophylaxis (Kasenga, Hurtig and Emmelin, 2007). In our setting interviews with midwives revealed a suboptimal knowledge on the recommended regimens and doses. Postpartum and postnatal prophylaxes are poorly implemented in sub-Saharan Africa (Mirkuzie, Hinderaker and Mørkve, 2010; Geddes *et al.*,

2011; Kirsten *et al.*, 2011). Likewise, data on postpartum prophylaxis was inexistent in our hospital and only 1 out of 29 newborns received correct postnatal prophylaxis. In case antenatal and intrapartum prophylaxes are correctly administered, between one-third and one-half of all mother-to-child transmission of HIV is estimated to occur during the postpartum period, mostly through breastfeeding (WHO, 2007b). Thus, a good and updated knowledge on postpartum and postnatal prophylaxes among staff working at maternity wards would make possible to maximize the chance of mother-baby pairs of getting appropriate and timely medications. However, frequent staff turnover and attendance by junior clinicians pose additional obstacles to the capacitation and continuous medical education of attending staff.

With Option B+ the differences between antenatal, intrapartum, and postpartum prophylaxes disappear. Since the moment the pregnant women is diagnosed to be HIV-positive, she is prescribed lifelong cART, including pregnancy, delivery and breastfeeding periods. This is much simpler than the current guidelines and the coming Option A. Thus, adopting Option B+ the gaps born from the complexity of the drug combinations of different periods for prophylaxis would be avoided, as well as many infant infections derived from the poor uptake of the more complex recommendations. Concerns about drug adherence have been raised due to the fact that some women will be asymptomatic and feel healthy. As seen in a Brazilian study, we expect pregnant women to be highly motivated to protect their babies, especially if they are periodically counseled and a simple regimen with not many pills per day is prescribed (WHO, 2007b). Extra counselling focused on minimizing the possibility of treatment interruption between pregnancies and its consequences might be necessary for asymptomatic mothers after the delivery and the breastfeeding period.

Similarly, postnatal prophylaxis would be simplified with Option B+. With this option, all HIV-exposed infants receive antiretroviral prophylaxes for four to six weeks, regardless of the feeding method. On the contrary, with the current recommendations and with the coming Option A, there are different regimens depending on the feeding method, which again add complexity to their implementation in the field.

Follow-up of HIV-exposed infants is notoriously weak in sub-Saharan Africa and it leads to under-diagnosis of HIV (Braitstein *et al.*, 2010), and our analysis confirms these findings in our setting. According to data from WHO, in 2010, only 28% of the HIV-exposed infants received an HIV test within the first two months of life among 65 reporting countries (WHO, 2011). Concurring with other studies from Kenya and Mozambique (Azcoaga-Lorenzo *et al.*, 2011; Cook *et al.*, 2011), a recent study from Ethiopia showed that slightly more than 50% of HIV-

exposed infants were followed-up at six weeks and less than one-third had documented an HIV test result. Remarkably, this low coverage of HIV testing occurred in the frame of an immunization coverage of more than 80% among HIV-exposed infants (Mirkuzie *et al.*, 2011), suggesting specific problems related with HIV testing. Integration of the follow-up of HIV-exposed infants into the existing under-five child health clinics would maximize the opportunities of getting a prompt diagnosis and timely treatment for HIV-infected children. Unfortunately, the lack of a standardized follow-up of HIV-exposed infants cannot directly be bridged by implementing Option B+. However, by providing Option B+ it is probable that the follow-up of HIV-exposed infants will improve, since more mothers will get medical care. Moreover, with this option, fewer infants are expected to be infected, so the number of undiagnosed infants will decrease.

Based on our findings several programmatic solutions can be proposed to increase the uptake of current and upcoming PMTCT guidelines. The implementation of the guidelines should be combined with a comprehensive package including educational and logistic interventions to be easily and affordably implemented in St. Francis referral hospital and other similar settings in sub-Saharan Africa. Analogously, Youngleson *et al.* implemented a "change package" to improve PMTCT program in a sub-district of Western Cape, South Africa, including maximizing the use of existing resources, reducing duplication, and developing patient-centered approaches. As a consequence of this program, significant improvements were achieved, with a decrease of the perinatal transmission rate from 7.6% to 5% (Youngleson *et al.*, 2010). The logistics of implementation would be easier if Option B+ was adopted, mainly due to a reduction on the training requirements. As a consequence the likelihood of a successful implementation would be greatly increased.

Furthermore, apart from the simplification of the PMTCT programme and its better implementation, lifelong cART for HIV-infected pregnant women (Option B+) has additional benefits. By adopting this strategy, future pregnancies will be protected since the conception, and sexual transmission of HIV to a serodiscordant partner will be significantly reduced (Cohen *et al.*, 2011). All women will require cART at some point, with Option B+ they will merely start it earlier. In this view, Option B+ can be placed in the frame of the idea of "treatment as prevention", which has garnered tremendous interest and hope (Cohen *et al.*, 2011; Shelton, 2011).

Conclusions

In summary, we strongly believe that it is time to move forward and argue in favor of Option B+ strategy in Tanzania and other sub-Saharan Africa settings. Option B+ is one of the most exciting developments in the prevention of vertical transmission and pediatric HIV in the recent years. Financial and operational challenges will have to be bridged and the feasibility, the cost-benefit and the public health impact will need to be assessed in those countries implementing Option B+. Data from solid scientific studies is still needed to definitively support Option B+. Reaching the WHO's call to the "virtual elimination" of pediatric HIV, aiming to attain a mother-to-child transmission risk of less than 5% for the year 2015, will be, even in the best scenario, hard to achieve. Option B+ gives us for the first time the opportunity to think that this goal is achievable at a global scale. Strong political will and strong support from governments and public health authorities will be needed to achieve this milestone in the history of the HIV pandemic.

6. *Study 2*: An Integrated and Comprehensive Service Delivery Model to Improve Pediatric and Maternal HIV Care in Rural Africa

J Acquir Immune Defic Syndr. 2016 Dec 15;73(5):e67-e75

An Integrated and Comprehensive Service Delivery Model to Improve Pediatric and Maternal HIV Care in Rural Africa

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Abstract

Background: Strategies to improve HIV diagnosis and linkage into care, antiretroviral treatment coverage and treatment outcomes of mothers and children are urgently needed in sub-Saharan Africa.

Methods: From 12/2012, we implemented an intervention package to improve Prevention of Mother-To-Child Transmission (PMTCT) and pediatric HIV care in our rural Tanzanian clinic, consisting of: a) creation of a PMTCT and pediatric unit integrated within the reproductive and child health clinic; b) implementation of electronic medical records; c) provider-initiated HIV testing and counseling in the hospital wards; and d) early infant diagnosis test performed locally. To assess the impact of this strategy, clinical characteristics and outcomes were compared between the period prior (2008-2012) and during/after the implementation (2013-2014).

Results: After the intervention, the number of mothers and children enrolled into care almost doubled. Compared to the pre-intervention period (2008-2012), in 2013-2014 children presented lower CD4% (16 vs. 16.8, p=0.08) and more advanced disease (WHO stage 3/4 72% vs. 35%, p<0.001). The antiretroviral treatment coverage rose from 80% to 98% (p<0.001), the lost to follow-up rate decreased from 20% to 11% (p=0.002) and mortality ascertainment improved. During 2013-2014, 261 HIV-exposed infants were enrolled and the early mother-to-child transmission rate among mother-infant pairs accessing PMTCT was 2%.

Conclusion: This strategy resulted in an increased number of mothers and children diagnosed and linked into care, a higher detection of children with AIDS, universal treatment coverage, lower loss to follow-up, and an early mother-to-child transmission rate below the threshold of elimination. This study documents a feasible and scalable model for family-centered HIV care in sub-Saharan Africa.

Introduction

Globally, 1.8 million children live with HIV (UNAIDS, 2016b), more than 90% of them residing in sub-Saharan Africa. Mother-To-Child Transmission (MTCT) accounts for over 90% of the infections (UNAIDS, 2013). Most of the newly infected children are found among those not reached by Prevention of MTCT (PMTCT) programs, and offering them timely diagnosis is vital. Systems to promote HIV testing for children outside the PMTCT programs need to be enhanced (Fergusson and Tomkins, 2009; Hesseling *et al.*, 2009; Cohen, Lungu and van Oosterhout, 2010; Kranzer *et al.*, 2014).

Antiretroviral treatment (ART) initiation and retention in care are essential for HIV-infected children (Newell *et al.*, 2004; Violari *et al.*, 2008; Cotton *et al.*, 2013). However, ART coverage among children in low and middle-income countries was only 25% in 2012 (UNAIDS, 2013). Training healthcare providers in pediatric HIV/AIDS is needed to bridge this gap, ensure high-quality programs and expand access to care and treatment among children (Kline *et al.*, 2009; Essajee *et al.*, 2013; Green *et al.*, 2014). Moreover, fragmentation of health services hinders the access to key populations, and integration of services is recommended (Tonwe-Gold *et al.*, 2009; Lindegren *et al.*, 2012; Gupta *et al.*, 2013; WHO, 2014b).

In 2014, the UNAIDS presented its "90-90-90 target" for 2020: i) 90% of people living with HIV will know their HIV status, ii) 90% of people with diagnosed HIV infection will receive ART, iii) 90% of people receiving ART will be virologically suppressed (UNAIDS, 2014a). In order to move towards this goal, special efforts need to be taken for the pediatric population given the challenges associated with timely diagnosis, ART coverage and retention in care.

In Tanzania, in 2013, 73% of HIV-infected pregnant women received PMTCT services and the estimated MTCT rate was 16% (UNAIDS, 2014), with 23,000 new pediatric infections (Tanzanian MoHSW, 2014). There are 250,000 children living with HIV, and ART coverage is dramatically lower than among adults, 26% versus 68% (UNAIDS, 2013).

Previous data from our clinic revealed several gaps in the care pathway of HIV-infected mothers and their children (Gamell *et al.*, 2013). In response to these challenges, we adopted a package of interventions to improve the care of HIV-infected children, mothers and their families attending our clinic.

The aim of this study was to evaluate the impact of this strategy on outcomes among both children and pregnant women over time.

Methods

This is a prospective cohort study evaluating the clinical outcomes of children and pregnant women before and during the implementation of a bundle of measures to improve the quality of HIV care in rural Tanzania.

Study setting and population

The Chronic Diseases Clinic of Ifakara is a rural HIV clinic in the Kilombero district, southern Tanzania. It is part of the Saint Francis Referral Hospital and works in cooperation with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute and the Department of Infectious Diseases and Hospital Epidemiology of the University Hospitals of Basel and Bern, Switzerland. All patients diagnosed with HIV within the hospital or diagnosed at a peripheral health centre and transferred for further treatment are referred to the clinic to receive HIV care and treatment according to the National AIDS Control Program. Since 2004, all HIV-infected patients attending the clinic are asked for informed consent to be enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO). This cohort study obtained ethical approval from the Ifakara Health Institute ethical review board, the Tanzanian National Institute for Medical Research, the Tanzanian Commission for Science and Technology and the Ethical Review Board of the Canton of Basel, Switzerland.

The cohort comprises more than 8,000 patients and is the largest rural HIV cohort in Tanzania . PMTCT Option B+ was implemented in April 2014 (Tanzanian MoHSW, 2013b).

In this study, we included all HIV-infected children (≤15 years) and pregnant women enrolled in the cohort between January 1, 2008 and December 31, 2014. For HIV-exposed infants, we included infants enrolled from January 1, 2013 until December 31, 2014, since no information was collected before this period.

Description of the intervention

From December 2012, a bundle of measures to improve the PMTCT and Pediatric HIV services were introduced, namely: a) creation of the One Stop Clinic of Ifakara: a PMTCT and Pediatric unit integrated within the Reproductive and Child Health Clinic; b) implementation of electronic medical records; c) implementation of provider-initiated HIV testing and counseling (PITC) in the hospital wards; and d) early infant HIV diagnosis (EID) test locally (See Table, Supplemental Digital Content 1).

Table, Supplemental Digital Content 1. Measures implemented to improve the care of HIV-infected pregnant women, children and their families

Year	Month	Implemented measure to improve the clinical care of pregnant women and children living with HIV and their families
2012	**December**	Introduction of new report forms to systematically and comprehensively collect clinical, drug and laboratory information
2013	**January**	Implementation of a standardized follow-up for HIV-exposed infants
	January - March	Creation of a PMTCT and Pediatric HIV clinical team
	April	Integration of PMTCT and HIV services for children and their families within the Reproductive and Child Health Clinic. Inauguration of the One Stop Clinic: antenatal, immunization, under-five and HIV services are offered the same day under the same roof.
	May	Launch of the electronic data collection system OpenMRS: the clinic becomes paperless
2014	**January**	Provider Initiated Testing and Counseling is implemented in the inpatient wards of the St Francis Referral Hospital
	March	Pro-viral HIV DNA PCR for Early Infant Diagnosis is established at the Ifakara Health Institute laboratory
	April	Implementation of PMTCT Option B+ guidelines in Ifakara
	July	A professional counselor joints the One Stop Clinic to coordinate all counseling activities including pre- and post-test, adherence, and disclosing sessions to HIV-infected children and adolescents, among others

PMTCT: Prevention of Mother-To-Child Transmission

a) Establishment of the One Stop Clinic of Ifakara

We created a unit to address the special needs of HIV-infected children and pregnant women. As in most HIV clinics in sub-Saharan Africa, prior to the intervention, infants, children and pregnant women were not assigned to specific clinicians and could be seen by different clinicians at each visit. Also, there was no family-centered approach and different family members were required to visit the clinic on different days. Additionally, clinicians based at our clinic were previously not in charge of the HIV-infected patients admitted in the hospital wards.

To establish and implement the One Stop Clinic, we pursued two steps.

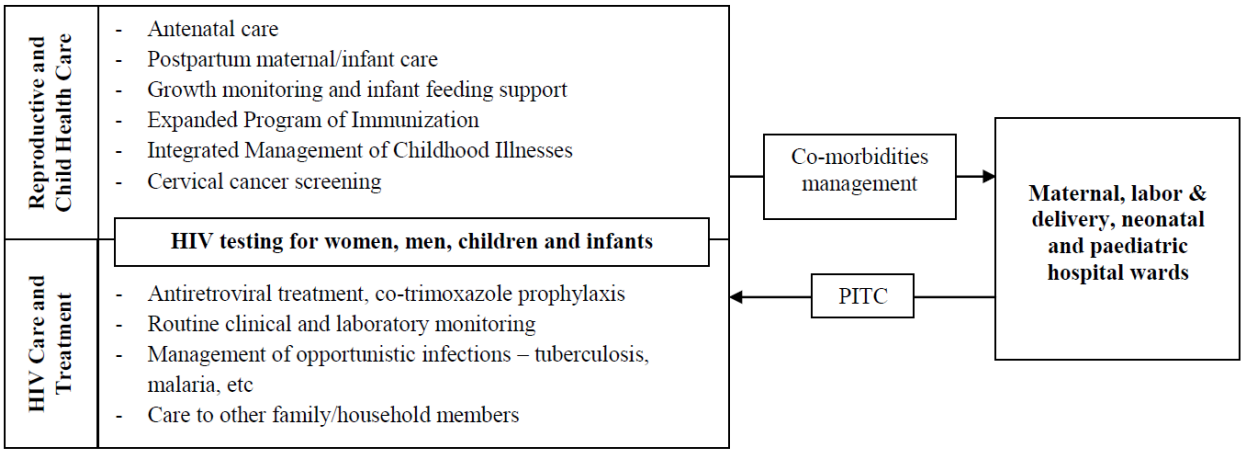
Step 1: Creation of a PMTCT and Pediatric HIV team

From January to March 2013, two clinicians, a nurse and a midwife were identified from the existing staff of the hospital. In July 2014 a counselor was newly hired and joined the team. All staff was provided with specific training and appointed to take care of HIV-infected children and pregnant women and HIV-exposed infants in both the outpatient clinic and the hospital wards.

Step 2: Integration within the Reproductive and Child Health Clinic

In April 2013 the care of HIV-infected children, pregnant women, HIV-exposed infants and their families was transferred to the Reproductive and Child Health Clinic. We intended to maximize the opportunities to diagnose women and children, facilitate their linkage into care, and improve retention by offering a range of services in a single day and place (Figure 1).

Figure 1. The One Stop Clinic of Ifakara integrates health services for HIV-infected children, pregnant women, HIV-exposed infants and their families in a rural Tanzanian hospital



PITC: Provider Initiated HIV Testing and Counseling

b) Electronic medical records for HIV-infected patients

The Open Medical Record System (OpenMRS, http://openmrs.org/) was adopted to implement an electronic data collection system at our clinic (Wolfe *et al.*, 2006). In December 2012 different questionnaires were introduced to systematically and comprehensively collect clinical, laboratory, and drug prescription information, and to minimize missing data and data entry mistakes. In May 2013 the clinic became paperless.

c) Provider-Initiated HIV Testing and Counseling

Provision of PITC had been recommended in Tanzania since 2007 but sub-optimally implemented in most settings, including our hospital (Tanzanian MoHSW, 2007a). In January 2014, our clinic joined efforts with the hospital administration to institutionalize PITC. Since then, all patients admitted in the pediatric and maternity wards are offered HIV testing through an opt-out strategy.

d) Early Infant Diagnosis Testing

Following the national recommendations, HIV-exposed infants are tested for HIV at 4-6 weeks of life or thereafter as soon as enrolled. Pro-viral DNA PCR is used for infants ≤9 months. For

those aged 9-18 months HIV rapid antibody tests are used for screening and, if positive, pro-viral DNA PCR is performed (Tanzanian MoHSW, 2013b).

Since March 2014 pro-viral HIV DNA PCR for EID is performed at the Ifakara Health Institute laboratory applying an in-house nested PCR protocol executed on a GeneAmp PCR System 9700 (Life Technologies, USA) using GoTaq DNA Polymerase (Promega, USA). Previously we had used the national EID circuit, which was often challenged by frequent stock outs of the kits to collect and store the samples and by the unreliable transport to one of the only four national referral laboratories.

Statistical analysis

Baseline characteristics of children and pregnant women were summarized according to year with appropriate statistical measures. Baseline is defined as the time of enrollment into the cohort.

To assess the impact of the interventions listed above, both the baseline characteristics and the clinical outcomes were compared in children and pregnant women separately between the period prior to the intervention (2008-2012) and during/after the implementation of the intervention (2013-2014). Baseline and follow-up comparisons between both periods were made using chi-square and Wilcoxon rank sum test for categorical and continuous variables respectively. Also, we compared the notified cases of tuberculosis and malnutrition between the time periods by estimating the overall Mantel-Haenszel rate ratio. Finally, we described the characteristics of the HIV-exposed infants enrolled and reported the early MTCT rate. Test statistics and 95% confidence intervals were presented. Data was analyzed using Stata version 13 (StataCorp, College Station, TX, USA).

Results

Cohort characteristics

A total of 200 HIV-infected pregnant women and 547 HIV-infected children were enrolled during the study period. Table 1 summarizes the baseline characteristics of the patients per year of enrolment. Pregnant women were enrolled at a median age of 30.3 years (interquartile range (IQR) 25.3-34.5), 20% were classified as WHO stage 3/4, and median CD4 was 340 cell/µL (IQR 147-494). Among children, 48% were female, with a median age of 4.4 years (IQR 1.5-8.3) and 48% presented with WHO stage 3/4. The median CD4 percent was 16.7% (IQR 8.7-28).

Table 1. Baseline characteristics of HIV-infected children and pregnant women newly enrolled in care from 2008 to 2014

	2008	2009	2010	2011	2012	2013	2014	Total
Pregnant women	**n=21**	**n=9**	**n=27**	**n=27**	**n=26**	**n=42**	**n=48**	**N=200**
Age, years, median (IQR)	30.7 (26.5, 35.9)	30.5 (26.6, 33.4)	29 (26.2, 34.9)	29.5 (25.6, 34.6)	29.6 (24.0, 33.9)	30.8 (24.8, 34.9)	29.9 (24.2, 34.1)	30.3 (25.3, 34.5)
WHO stage (%)[a]								
1 or 2	12 (57)	7 (78)	23 (88)	25 (96)	23 (92)	30 (71)	37 (77)	157 (80)
3 or 4	9 (43)	2 (23)	3 (12)	1 (4)	2 (8)	12 (29)	11 (23)	40 (20)
Absolute CD4 counts, median (IQR) [b]	318 (228, 499)	164 (114, 520)	368 (310, 513)	491 (317, 556)	390 (75, 741)	290 (48, 454)	335 (142, 450)	340 (147, 494)
Children	**n=127**	**n=126**	**n=49**	**n=45**	**n=56**	**n=54**	**n=90**	**N=547**
Female (%)	56 (44)	63 (50)	24 (49)	21 (47)	31 (55)	23 (43)	45 (50)	263 (48)
Age, years, median (IQR)	5.7 (1.9, 9.5)	3.1 (1.3, 6.6)	4.1 (1.1, 8.3)	4.4 (1.9, 8.0)	5.1 (1.8, 9.4)	2.5 (1.3, 7.9)	4.2 (1.2, 8.3)	4.4 (1.5, 8.3)
WHO stage (%)[c]								
1 or 2	81 (65)	86 (72)	27 (61)	20 (45)	24 (50)	8 (17)	23 (26)	269 (52)
3 or 4	43 (35)	33 (28)	17 (39)	24 (55)	24 (50)	39 (83)	65 (74)	245 (48)
Absolute CD4 counts, median (IQR) [d]	490 (192, 848)	565 (263, 1145)	576 (108, 1142)	424 (24, 1061)	473 (172, 549)	493 (187, 805)	688 (338, 1086)	518 (192, 979)
Percent CD4 , median (IQR) [e]	14.9 (4.4, 21.7)	20.2 (9.9, 35.4)	15.6 (7.1, 34.1)	32.0 (14.8, 64.4)	14.7 (11.0, 21.8)	13.0 (5.0, 21.0)	18.0 (10.0, 26.0)	16.7 (8.7, 28)

[a] WHO stage missing for 3 pregnant women
[b] Absolute CD4 counts missing for 58 pregnant women
[c] WHO stage missing for 33 children
[d] Absolute CD4 counts missing for 156 children
[e] Percent CD4 missing for 164 children
IQR: interquartile range; WHO: World Health Organization

Outcomes of pregnant women

The median number of women enrolled per year increased from 26 (IQR 21-27) in 2008-2012 to 45 (IQR 42-48) in 2013-2014 (p=0.05) (Figure 2A). After the intervention, there was a trend towards a higher WHO stage 3/4 (26% in 2013-2014 versus 15% in 2008-2012, p=0.08) and lower CD4 counts (median 315 cells/µL versus 370 cells/µL, p=0.06). ART coverage among those eligible was above 98% during the whole study period. In 2013-2014, most women were initiated on a fixed-dose combination of tenofovir/lamivudine or emtricitabine/efavirenz, as expected due to the changes of the national guidelines. There were no major differences in retention in care, although lost to follow-up declined after 2012 (26.5% versus 30.9%) (Table 2).

Table 2. Comparison of the clinical characteristics, antiretroviral treatment coverage and regimens, and retention in care of patients enrolled before and after the intervention

	2008 - 2012	2013 - 2014	Effect Estimate	p-value
Pregnant women	**n=110**	**n=90**		
WHO stage (%)			3.16[b]	0.08
1 or 2	93 (84.6%)	67 (74.4%)		
3 or 4	17 (15.5%)	23 (25.6%)		
Absolute CD4 counts, median (IQR)	370 (178-556)	315 (117-450)	1.91[a]	0.06
ART coverage [d], n/N (%)	51/52 (98.1%)	68/69 (98.6%)	0.04[b]	0.84
Time to ART initiation [e], days, median (IQR)	0 (0-4)	1.5 (0-10)	-1.81[a]	0.07
Initial ART regimen (%)			96.32[b]	<0.001
D4T/3TC/NVP or AZT/3TC/NVP	36 (46.8%)	3 (4.7%)		
AZT/3TC/EFV	31 (40.3%)	2 (3.1%)		
TDF/FTC/EFV [g] or TDF/3TC/EFV [h]	9 (11.7%)	58 (90.6%)		
PI - based regimen	0	1 (1.6%)		
Retention in care 6 months after enrolment [f]			1.23[i]	0.68
Lost to follow-up	34 (30.9%)	18 (26.5%)		
Died	1 (0.9%)	1 (1.5%)		
Transferred to another clinic	7 (6.4%)	7 (10.3%)		
Active follow-up	68 (61.8%)	42 (61.8%)		
Children	**n=403**	**n=144**		
Age, years, median (IQR)	4.5 (1.6 – 8.6)	3.2 (1.3 – 8.1)	1.41[a]	0.16
Referred from				
Voluntary testing	307 (76.2%)	71 (49.3%)	35.88[b]	<0.001
Provider initiated testing	41 (10.2%)	25 (17.4)	5.12 [b]	0.02
PMTCT / EID Program	9 (2.3%)	8 (6.4%)		0.04[i]
Transferred from other facilities	19 (4.8%)	24 (19.2%)	26.24 [b]	<0.001
WHO stage			59.48[b]	<0.001
1 or 2	262 (65.0%)	40 (27.8%)		
3 or 4	141 (35.0%)	104 (72.2%)		
Percent CD4, median (IQR)	16.8 (8.7-30.0)	16 (9-22)	1.73[a]	0.08
Absolute CD4 counts, median (IQR)	490 (170-906)	560 (264-1053)	-1.39[a]	0.16
Tuberculosis				
Cases notified	72	53	3.96 (2.7-5.9)[c]	<0.001
Incidence (per 100 persons-years)	12.18	103.09		
Malnutrition				
Cases notified	23	67	11.22 (5.9-21.5)[c]	<0.001
Incidence (per 100 persons-years)	4.13	145.90		
% cases severe malnutrition	26.1	62.7		
ART coverage [d], n/N (%)	215/270 (79.6%)	101/103 (98.1%)	19.56[b]	<0.001

Time to ART initiation [e], days, median (IQR)	1 (0-16)	11 (2-19)	-2.89[a]	0.004
Initial ART regimen (%)			43.93[b]	<0.001
NVP-based regimen	175 (60.1%)	48 (47.5%)		
EFV- based regimen	114 (39.2%)	36 (35.6%)		
PI - based regimen	2 (0.7%)	17 (16.8%)		
Retention in care 6 months after enrolment [f]			15.29[b]	0.002
Lost to follow-up	82 (20.4%)	11 (10.8%)		
Died	23 (5.7%)	16 (15.7%)		
Transferred to another clinic	26 (6.5%)	9 (8.8%)		
Active follow-up	272 (67.5%)	66 (64.7%)		

[a] Z-score from a two-sample Wilcoxon rank-sum test
[b] Pearson chi-square test of association
[c] Mantel-Haenszel estimates of the rate ratio and 95% confidence interval
[d] ART coverage: patients meeting criteria to start and starting within 90 days. Excludes patients who started ART prior to meeting criteria or those starting ART without meeting criteria.
[e] Days between meeting criteria to start ART and ART initiation in those starting within 90 days. Excludes patients who started ART prior to meeting criteria or those starting ART without meeting criteria.
[f] N=42 paediatric patients and n=22 pregnant women in the period after 2013 were not under care long enough be evaluated for retention in care.
[g] First available in 2009
[h] First available in 2013
[i] Fisher's exact test
[j] In those who were not diagnosed with severe malnutrition

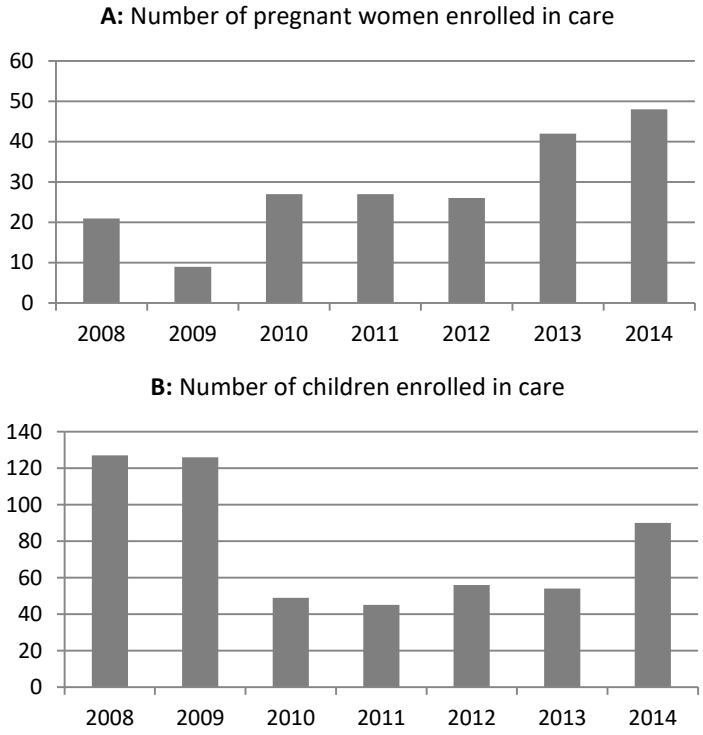
WHO: World Health Organization; IQR: Interquartile range; ART: antiretroviral treatment; D4T: stavudine, 3TC: lamivudine, NVP: nevirapine; AZT: zidovudine; EFV: efavirenz; FTC: emtricitabine; PI: protease inhibitor; PMTCT: Prevention of Mother-To-Child Transmission; EID: Early Infant Diagnosis.

Outcomes of children

The number of children enrolled peaked in 2008 and 2009, following the national trends (Tanzanian MoHSW, 2013a), and increased sharply in 2014 in opposition to the tendency in the country (Figure 2B). As shown in Table 2, after the intervention, the proportion of children diagnosed through PITC, PMTCT/EID program, and transferred from other facilities increased significantly. In 2013-2014, children were enrolled with more advanced disease (WHO stage 3/4 72.2% versus 35%, p<0.001) and immunosupression (median CD4 percent 16 versus 16.8, p=0.08). In total, 125 cases of tuberculosis were notified during the study period, 53 of them during 2013-2014, representing an 8.5-fold increase of the incidence of tuberculosis ascertainment, from 12.2 in 2008-2012 to 103.1 per 100 person-years in 2013-2014. The thorough nutritional assessment was reflected in the 35.6-fold increased incidence of malnutrition ascertainment, from 4.1 in 2008-2012 to 145.9 per 100 person-years in 2013-2014. After the intervention, ART coverage increased from 79.6% to 98.1% (p<0.001). The time between meeting criteria to start treatment and ART initiation increased from one to eleven days (p=0.004). Children prescribed protease inhibitor-based regimens increased from 0.7% to 16.8% (p<0.001) as a result of the adoption of the 2010 WHO ART guidelines by Tanzania in 2012 combined with the increased diagnosis of children through the PMTCT/EID program. The lost to follow-up and documented mortality rates varied largely during the two periods. While the lost to follow-up rate decreased from 20.4% to 10.8%, the documented mortality increased

from 5.7% to 15.7%. However, remarkably all deaths in 2013-2014 happened among children diagnosed through PITC: 12/16 in the inpatient wards, 2/16 in the tuberculosis clinic, and 2/16 in the outpatient department. Fifteen (15/16) were malnourished, 6/16 had tuberculosis and all died within 90 days after diagnosis, the majority (10/16) within six weeks.

Figure 2. Number of newly enrolled pregnant women (A) and children (B) per year



Characteristics of the HIV-exposed infants and early Mother-To-Child Transmission

Since January 2013 all HIV-exposed infants <18 months are enrolled in our clinic. From January 1, 2013 to December 31, 2014, 261 infants were enrolled. Table 3 summarizes the characteristics of these infants. Fifty-six percent (157/261) of the mothers were diagnosed before pregnancy, 33.3% (87/261) during pregnancy or delivery, and 10.3% (27/261) after delivery. Among the mothers diagnosed before or during pregnancy, 15.4% (36/234) did not receive a correct PMTCT intervention, all recruited before the integration of services. The overall early MTCT rate, defined as the rate of positivity of the first HIV test performed, was 8.6%. Yet, it varied largely depending on the timing of the HIV diagnosis of the mother and the correctness of PMTCT (See Figure, Supplemental Digital Content 2). The MTCT rate of mother-infant pairs diagnosed before or during pregnancy was 4.8% (11/231), being as low as 2% (4/195) for the 84.6% (195/234) of pairs who received a correct PMTCT intervention. However, the rate was 44% (11/25) for pairs diagnosed after delivery. Noteworthy, many infants in this last group were found to be HIV-exposed through PITC, when admitted with symptoms. By the

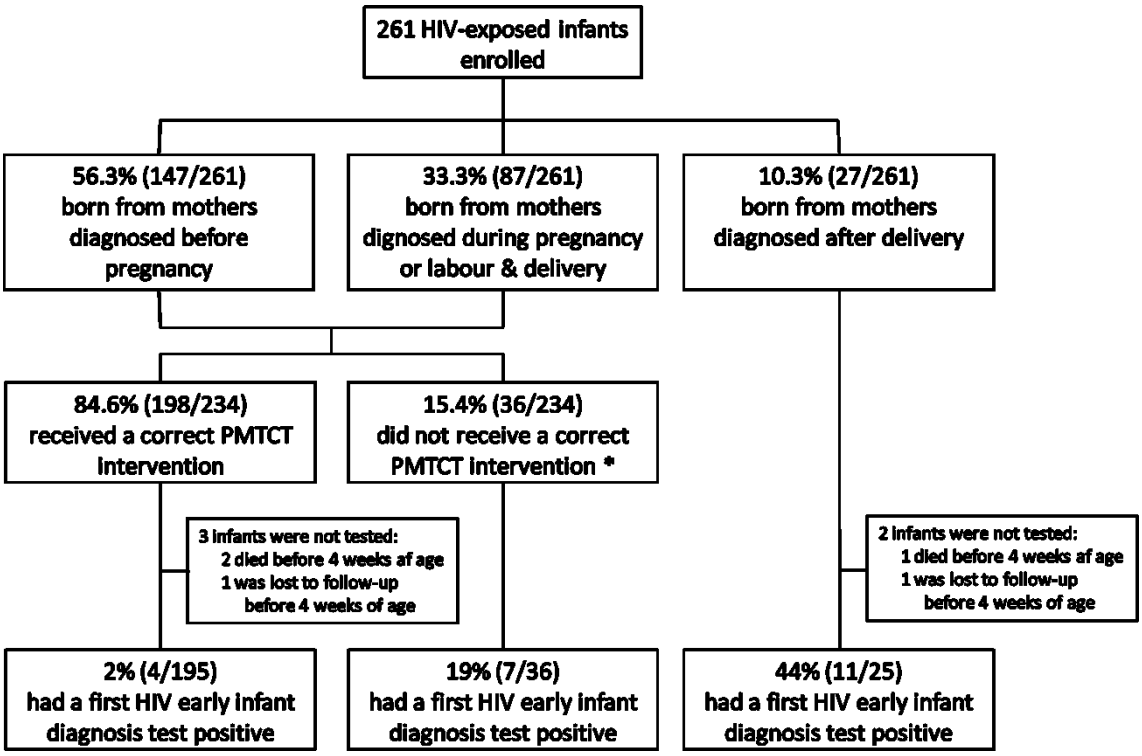
time of analysis, none of the breastfeeding infants with an initial negative test had seroconverted.

Table 3. Characteristics of the 261 HIV-exposed infants enrolled during 2013 - 2014

Characteristic	
Female gender, n/N (%)	120/261 (46.2)
Age at enrolment, weeks, median (IQR)	6 (5-14)
Time of mother's HIV diagnosis, n/N (%)	
Before pregnancy	147/261 (56.3)
During pregnancy, labour or delivery	87/261 (33.3)
After delivery	27/261 (10.3)
Correct PMTCT intervention [a], n/N (%)	198/234 (84.6)
Timely initiation of co-trimoxazole prophylaxis [b], n/N (%)	171/239 (71.6)
Feeding practice during first 6 months of life n/N (%)	
Exclusive breastfeeding	236/261 (90.4)
Formula milk	8/261 (3.1)
Animal's milk	4/261 (1.5)
Mixed feeding	13/261 (5.0)
Age at first early infant diagnosis test, weeks, median (IQR)	7 (6-16)
Result of first HIV test performed, n/N [c] (%)	
Negative	234/256 (91.4)
Positive	22/256 (8.6)
Final HIV serostatus, n/N (%)	
Not yet known	163/256 (63.7)
Uninfected	76/256 (29.7)
Infected	22/256 (8.6)
Correct PMTCT intervention	4
Incorrect/incomplete PMTCT intervention	7
No PMTCT intervention (mother diagnosed after delivery)	11
Retention in care, n/N (%)	
Retained	193/261 (74.0)
Dead [d]	19/261 (7.3)
Transferred to another clinic	16/261 (6.1)
Lost to follow-up [e]	33/261 (12.6)

[a] Correctness according to the national PMTCT guidelines applicable during the pregnancy. Applicable only for mother-infant pairs diagnosed before or during pregnancy, labour or delivery (n = 234).
[b] Infants initiating prophylaxis between 4 and 8 weeks of life. We include 239 infants: 231 born from mothers diagnosed before or during pregnancy, labour or delivery and 8 infants born from mothers diagnosed after delivery and before the infant was 8 weeks old. Three infants born from mothers diagnosed before or during pregnancy died or were lost to follow-up before 4 weeks of age and were excluded.
[c] Five infants were never tested with pro-viral HIV DNA PCR: 3 died before 4 weeks of age and 2 were lost to follow-up before 4 weeks of age.
[d] Six of these infants were HIV-infected
[e] Lost to follow-up was defined as > 90 days had passed since their next scheduled visit

Figure, Supplemental Digital Content 2. Early mother-to-child transmission rate among HIV-exposed infants enrolled during 2013-2014



*All the mothers in this group were attended before the HIV care and treatment services were integrated within the Reproductive and Child Health Clinic
PMTCT: Prevention of Mother-To-Child Transmission

After EID was implemented in Ifakara in March 2014, the turnaround time for results -time between sample collection and delivery of results to caregivers - decreased from 7 months in 2013, to 13 and 35 days for positive and negative results, respectively.

Discussion

The implementation of the One Stop Clinic of Ifakara combined with the improved efficiency of a paperless clinic and the rollout of PITC and EID resulted in: a) an increased number of pregnant women and children diagnosed and linked into care; b) an increased detection of children with AIDS; c) universal ART coverage; d) lower loss to follow-up and better ascertainment of mortality; and e) a proof that elimination of MTCT can be achieved in rural Tanzania.

The intervention resulted in an increased number of pregnant women and children diagnosed and linked into care. This is an effect of the active case-finding approach of our strategy and the presence of a PMTCT and Pediatric HIV team, which is well-integrated and coordinated with the outpatient clinic and the hospital wards. The opt-out approach for HIV testing during antenatal care visits has been in place in our hospital since 2008. However, in 2010-11 only

25% of newly diagnosed women were linked to HIV services (Gamell *et al.*, 2013). Through the One Stop Clinic the number of women diagnosed and linked into care increased substantially, with the most likely impact of preventing new pediatric infections.

Diagnosing children outside the PMTCT programs remains challenging (Horwood *et al.*, 2010; Ahmed *et al.*, 2013; Chamla *et al.*, 2013). Measures to identify HIV-infected children must be implemented in parallel to the efforts to achieve universal PMTCT and the elimination of MTCT. In our study, the increased number of children enrolled in 2014, was mainly attributable to PITC but also to the enrollment of children diagnosed in other facilities and admitted with severe disease and the implementation of EID locally. Also, it is possible that as a result of an increased awareness among the staff, there was an expansion of family-centered testing using parents as index cases. Children enrolled after 2012 presented with more advance disease and immunosuppression, reflecting the high yield of infants and children enrolled from the inpatient wards (Kankasa *et al.*, 2009; McCollum *et al.*, 2010; Mutanga *et al.*, 2012) and probably a better clinical assessment by a specially trained team (Kline *et al.*, 2009). The incidence of HIV has decreased over the last years in Tanzania (UNAIDS, 2013). Thus, we presume that before the intervention, some children were admitted and eventually died without being diagnosed with HIV and therefore enrolled in our cohort. The improvement of EID is expected to increase the identification of asymptomatic HIV-infected infants. However, given the low MTCT among mother-infant pairs that received an appropriate PMTCT intervention, the few infants diagnosed during the routine follow-up did not have an impact on the proportion of children with advanced disease at diagnosis. On the contrary, the implementation of local HIV DNA PCR testing allowed us to confidentially diagnose children < 18 months admitted in the hospital with advance disease. Diagnosing and offering health-restoring care and treatment to children with AIDS is one of the major achievements of our strategy. However, it highlights the high percentage of late presenters in sub-Saharan Africa (Lahuerta *et al.*, 2014) and the need to expand the case-finding approaches to places where large number of infants and children congregate, such as immunization clinics, schools and the community, to increase early diagnosis and improve outcomes (Ahmed *et al.*, 2013).

ART coverage among eligible children increased from 79.6% to 98.1%. This achievement is especially remarkable since, to date, similar coverage rates in Africa have only been reported by urban programs (Anaky *et al.*, 2010; Leyenaar *et al.*, 2010). For children meeting criteria, the time to initiation of drugs increased from one to eleven days, likely reflecting a better

ascertainment of opportunistic infections and improved pre-ART counseling to children and caregivers.

After initiated on ART, children need to be retained, adhere to treatment and get the support needed to face the challenges of growing with HIV (Kellerman and Sugandhi, 2013; Bernays *et al.*, 2014; Lowenthal *et al.*, 2014). Integration of PMTCT and Pediatric HIV services with reproductive and child care, and family-centered approaches have proven effective to improve retention (Myer and Akugizibwe, 2009; Rochat *et al.*, 2011; Essajee *et al.*, 2013). In our cohort, the lost to follow-up rate decreased from 20.4% to 10.8%. However, we could not fully assess the contribution of mortality to these cases (Geng *et al.*, 2015). The increased documented mortality after the intervention can be explained by the increased number of children with AIDS and a better ascertainment of death. Known risk factors for mortality are low CD4 percent, WHO stage 3/4 disease, severe malnutrition and tuberculosis (Eley *et al.*, 2006; Bong *et al.*, 2007; Sutcliffe *et al.*, 2008), all common among children registered in 2013-14. High fatality rates have been reported in pediatric African cohorts. In Malawi, the 12-months mortality after diagnosis through PITC was 20% (McCollum *et al.*, 2011), same as for children with tuberculosis-HIV co-infection (Buck *et al.*, 2013). In Zambia, mortality of malnourished children was 46%, with HIV-infected children being 80% more likely to die (Munthali *et al.*, 2015). Although the lost to follow-up rate decreased in our clinic, the reported early mortality emphasizes once more the need for strategies to timely diagnose children that fell through the cracks of the PMTCT programs. Moreover, the availability of pediatric antiretroviral formulations is likely to lead to further improvements in adherence and retention (DNDi, 2013).

The MTCT rate for mother-infant pairs reached by PMTCT in our clinic achieved the goal of virtual elimination (<5%). These low transmission rates combined with the finding that all mothers attended after the establishment of the One Stop Clinic received a complete PMTCT intervention confirm the programmatic benefits of integrating services. The dramatic reduction of the turnaround time of EID results leverages the decentralization of this test in order to facilitate the timely initiation of life-saving treatment for infected infants (Violari *et al.*, 2008).

This study has some limitations. First, although we believe that given the comprehensive nature of the intervention, its positive impact has a good chance to be maintained over time, a longer follow-up will be needed to measure the long-term impact of the strategy on the clinical outcomes and retention in care. Second, it is challenging to distinguish the impact of the

specific interventions, as they were implemented as a bundle. Third, tuberculosis might have been over-diagnosed, since, despite having Xpert MTB/RIF (Cepheid, Sunnyvale, CA) in our clinic (Haraka *et al.*, 2015), confirming childhood tuberculosis is challenging and most diagnoses were based on symptoms and radiological findings. Forth, it is possible that some cases ascertained as lost to follow-up had died. Finally, given the short follow-up time, we could not assess the final MTCT rate. Strengths of our study include the comprehensive clinical data and the prospective character of the evaluation.

In conclusion, the creation of One Stop Clinic of Ifakara combined with a comprehensive electronic data collection system and the implementation of PITC and EID resulted in an increased number of mothers and children diagnosed and linked into care, a higher detection of children with AIDS, universal ART coverage, better retention in care and ascertainment of mortality, and an early MTCT rate below the elimination threshold. This strategy may provide a feasible and scalable model for delivering high-quality family-centered HIV care in Tanzania and achieve the 90-90-90 target.

Acknowledgments

The KIULARCO Study Group: Aschola Asantiel, Adolphina Chale, Diana Faini, Ingrid Felger, Gideon Francis, Hansjakob Furrer, Speciosa Hwaya, Aneth Kalinjuma, Bryson Kasuga, Namvua Kimera, Yassin Kisunga, Thomas Klimkait, Antonia Luhombero, Leticia Mbwile, Mengi Mkulila, Julius Mkumbo, MargarethMkusa, Dorcas K Mnzava, Germana Mossad, Dolores Mpundunga, Daimon Msami, Athumani Mtandanguo, Selerine Myeya, Sanula Nahota, Regina Ndaki, Agatha Ngulukila, George Sikalengo, Fiona Vanobberghen, and Maja Weisser.

The authors are grateful to all the children and families attended in the One Stop Clinic and the staff from the Saint Francis Referral Hospital in Ifakara. Also, we want to thank the funders of our clinic: the Ministry of Health and Social Welfare of Tanzania, the Swiss Tropical and Public Health Institute, the Ifakara Health Institute, the Government of the Canton of Basel, USAID through the local implementer TUNAJALI-Deloitte, and the Merck for Mothers Global Giving Program.

7. *Study 3*: Prevention of Mother-To-Child Transmission Option B+ Cascade in Rural Tanzania: the One Stop Clinic Model

PLoS One. 2017 Jul 12;12(7):e0181096.

Prevention of Mother-To-Child Transmission of HIV Option B+ Cascade in Rural Tanzania: the One Stop Clinic Model

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Abstract

Background: Strategies to improve the uptake of Prevention of Mother-To-Child Transmission of HIV (PMTCT) are needed. We integrated HIV and maternal, newborn and child health services in a One Stop Clinic to improve the PMTCT cascade in a rural Tanzanian setting.

Methods: The One Stop Clinic of Ifakara offers integral care to HIV-infected pregnant women and their families at one single place and time. All pregnant women and HIV-exposed infants attended during the first year of Option B+ implementation (04/2014-03/2015) were included. PMTCT was assessed at the antenatal clinic (ANC), HIV care and labour ward, and compared with the pre-B+ period. We also characterised HIV-infected pregnant women and evaluated the MTCT rate.

Results: 1,579 women attended the ANC. Seven (0.4%) were known to be HIV-infected. Of the remainder, 98.5% (1,548/1,572) were offered an HIV test, 94% (1,456/1,548) accepted and 38 (2.6%) tested HIV-positive. 51 were re-screened for HIV during late pregnancy and one had seroconverted. The HIV prevalence at the ANC was 3.1% (46/1,463). Of the 39 newly diagnosed women, 35 (90%) were linked to care. HIV test was offered to >98% of ANC clients during both pre- and post-B+ periods. During the post-B+ period, test acceptance (94% versus 90.5%, $p<0.0001$) and linkage to care (90% versus 26%, $p<0.0001$) increased. Ten additional women diagnosed outside the ANC were linked to care. 82% (37/45) of these newly-enrolled women started antiretroviral treatment (ART). After a median time of 17 months, 27% (12/45) were lost to follow-up. 79 women under HIV care became pregnant and all received ART. After a median follow-up time of 19 months, 6% (5/79) had been lost. 5,727 women delivered at the hospital, 20% (1,155/5,727) had unknown HIV serostatus. Of these, 30% (345/1,155) were tested for HIV, and 18/345 (5.2%) were HIV-positive. Compared to the pre-B+ period more women were tested during labour (30% versus 2.4%, $p<0.0001$). During the study, the MTCT rate was 2.2%.

Conclusions: The implementation of Option B+ through an integrated service delivery model resulted in universal HIV testing in the ANC, high rates of linkage to care, and MTCT below the elimination threshold. However, HIV testing in late pregnancy and labour, and retention during early ART need to be improved.

Introduction

Mother-To-Child Transmission (MTCT) accounts for over 90% of new paediatric HIV infections (UNAIDS, 2010b). The World Health Organization (WHO) has issued several prevention of MTCT (PMTCT) recommendations for low and middle-income countries since 2001 (WHO, 2004, 2012b). As a result of the scale-up of PMTCT interventions, there has been a 70% decline of new HIV infections among children between 2000 and 2015. However, in 2015, 23% of HIV-infected pregnant women did not receive effective antiretroviral regimens for PMTCT and 150,000 children acquired HIV (UNAIDS, 2016b).

Since 2012, the WHO recommends using lifelong antiretroviral therapy (ART) for all pregnant and breastfeeding women regardless of CD4 counts and clinical stage, and provision of nevirapine or zidovudine to all HIV-exposed infants for four to six weeks regardless of the feeding method. These recommendations are known as "Option B+".

In Tanzania, Option B+ was rolled out from September 2013 (Tanzanian MoHSW, 2013b). The MTCT rate in 2014 was 9% (UNAIDS, 2015a). In the Saint Francis Referral Hospital (SFRH) of Ifakara, in rural south-west Tanzania, Option B+ was deployed in April 2014. In 2012, an assessment of the PMTCT circuit had identified several gaps, namely: (a) poor linkage into HIV care of newly diagnosed HIV-infected pregnant women; (b) no re-testing of seronegative women in late pregnancy; and (c) lack of a standardised follow-up of HIV-exposed infants (Gamell *et al.*, 2013). To bridge these gaps, an integrated and comprehensive service delivery model to improve maternal and paediatric HIV care was implemented in parallel to Option B+ (Gamell, Glass, *et al.*, 2016; Muri *et al.*, 2016).

The current study describes the PMTCT cascade and uptake of Option B+ guidelines implemented through this service delivery model.

Methods

This is a prospective cohort study describing the PMTCT cascade and uptake of Option B+ guidelines in the SFRH. The uptake of PMTCT recommendations was compared with the one previously described in 2012 (Gamell *et al.*, 2013). We also assessed the MTCT rate, characterised HIV-infected mothers and analyzed the differences between newly diagnosed pregnant women and women who became pregnant while being under HIV care.

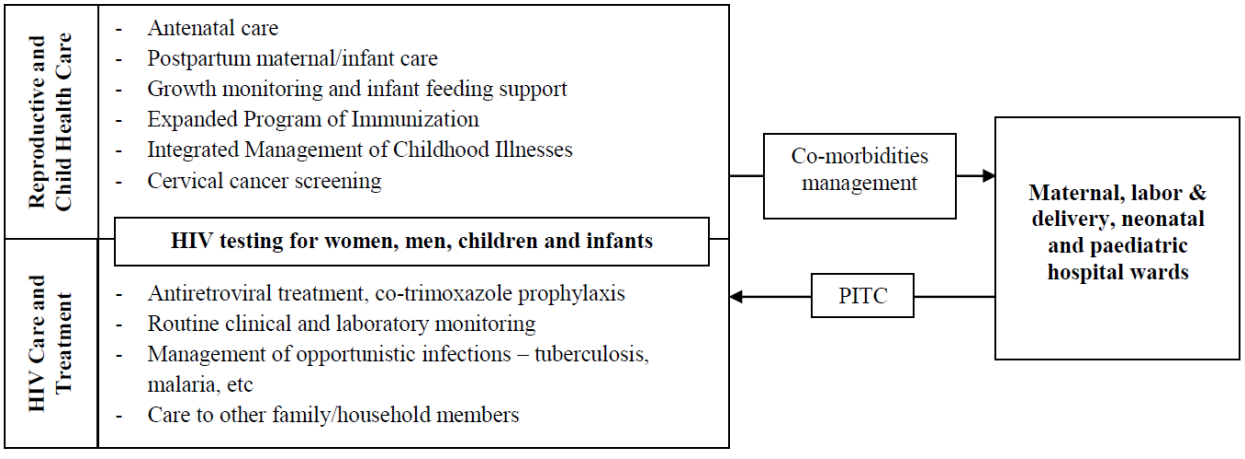
Study setting and population

The SFRH is the largest healthcare facility in the rural Kilombero district, serving a population of 600,000 inhabitants and an estimated 30,000 people living with HIV (Tanzanian NBS, 2012). HIV care and treatment is offered within the hospital at the Chronic Diseases Clinic of Ifakara

(CDCI). The clinic works in collaboration with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute and the Department of Infectious Diseases and Hospital Epidemiology of the University Hospitals of Basel and Bern, Switzerland. Since 2004, all patients attending the clinic are invited to participate in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) (Haraka *et al.*, 2015; Letang *et al.*, 2015; Ramírez-Mena *et al.*, 2016). Written informed consent is sought from all participants; for children and adolescents aged < 18 years, informed consent is sought from caregivers. The KIULARCO study obtained ethical approval from the Ifakara Health Institute ethical review board, the National Institute for Medical Research of Tanzania, the Tanzanian Commission for Science and Technology and the ethical review board of Northwest Switzerland. It is the largest rural HIV cohort in Tanzania, with over 9,000 patients ever enrolled. Patients receive care according to the National AIDS Control Program (Tanzanian MoHSW, 2015).

In December 2012 the One Stop Clinic of Ifakara was created, a maternal and paediatric family-oriented HIV unit integrated within the Reproductive and Child Health Clinic. The services delivered include: antenatal care (ANC), postpartum maternal and infant care, cervical cancer screening, child's growth and health monitoring, expanded program of immunizations, nutritional assessment and tuberculosis and HIV care and treatment (Figure 1). Details about the organization, functioning and staff of the One Stop Clinic have been published elsewhere (Gamell, Glass, *et al.*, 2016; Letang *et al.*, 2017).

Figure 1: The One Stop Clinic of Ifakara integrates health services for HIV-infected pregnant women, children, HIV-exposed infants and their families in a rural Tanzanian hospital



PITC: Provider Initiated HIV Testing and Counseling

In this study we included all pregnant women and HIV-exposed infants attended in the ANC station, labour ward and/or the CDCI in SFRH during the first year of Option B+

implementation, from April 1st, 2014 to March 31st, 2015. Data from HIV-infected pregnant women was analysed by February 11th, 2016. For HIV-exposed infants, given the long breastfeeding period, retention in care and final HIV serostatus was analysed as per June 30th, 2016. HIV-infected pregnant women transferred to the One Stop Clinic from other facilities were excluded. Lost to follow-up was defined as not having visited the clinic 60 days after the last scheduled date for HIV-infected mothers and 90 days for HIV-exposed infants.

Statistical analysis

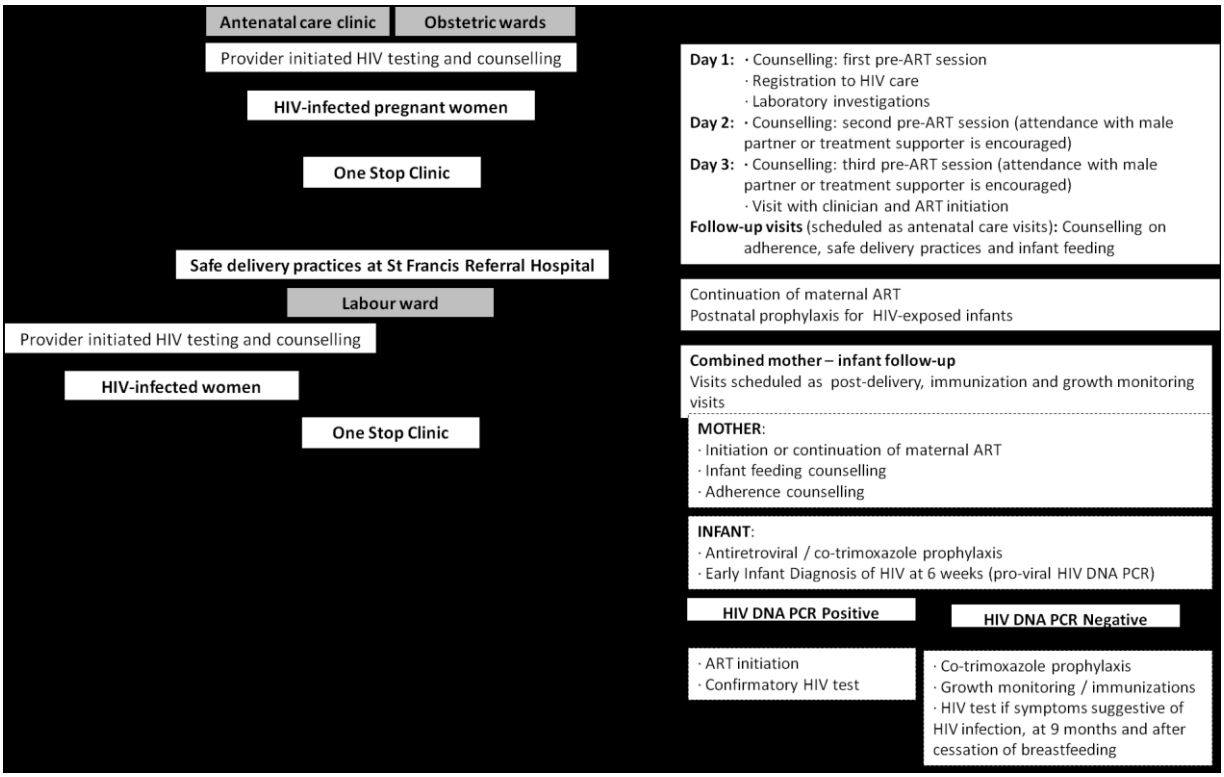
Characteristics of pregnant women were summarised using proportions and medians. The comparison of data from ANC and labour ward during the pre-B+ and post-B+ periods was done using proportion differences. Comparisons between newly enrolled pregnant women and women who became pregnant while being under care were made using Chi-square and Wilcoxon rank-sum tests for categorical and continuous variables, respectively. Fisher exact test was used to compare proportions of women's follow-up status. Finally, we described the characteristics of the HIV-exposed infants enrolled and reported the MTCT rate. SAS 9.3 was used for data analysis (SAS Institute Inc., Cary, NC, USA).

Results

Implementation of a new PMTCT care pathway

Since 2013 comprehensive PMTCT services are delivered within the Reproductive and Child Health Clinic (WHO, 2012b; Tanzanian MoHSW, 2013b) and coordinated by the One Stop Clinic. These services involve different hospital units: the ANC, the HIV clinic and the labour ward. Option B+ services include: 1) routine HIV counselling and testing for pregnant women and male partners; 2) comprehensive antenatal care; 3) lifelong ART for HIV-positive pregnant and breastfeeding women; 4) safe delivery practices; 5) postpartum care for mothers and infants; 6) antiretroviral and co-trimoxazole prophylaxis for HIV-exposed infants; 7) counselling for safe infant feeding practices; 8) early infant HIV diagnosis and treatment for HIV-infected infants; 9) male partner and family involvement. Figure 2 illustrates the PMTCT care pathway at SFRH.

Figure 2: Current Prevention of Mother-To-Child Transmission of HIV care pathway at Saint Francis Referral Hospital



ART: antiretroviral treatment

PMTCT at the Antenatal Clinic

From April 1st, 2014 to March 31st, 2015, 1,579 women attended the ANC and 0.44% (7/1,579) were previously known to be HIV-infected. Ninety-eight percent of women with unknown HIV serostatus (1,548/1,572) were offered an HIV test and 94% (1,456/1,548) accepted. Thirty-eight (38/1,456, 2.6%) were found to be HIV-positive. Among the seronegative women 3.6% (51/1,418) were re-tested for HIV during late pregnancy and one (2%) had seroconverted. In total, 46 women were identified as HIV-infected in the ANC, for an overall HIV prevalence of 3.1% (46/1,463). Compared to the period January 2010 – December 2011, acceptance of HIV testing improved, (94% versus 90.5% %) after Option B+ implementation ($p<0.0001$), and the HIV prevalence at the ANC decreased from 6.9% to 3.1% ($p<0.0001$; Table 1).

Table 1: Changes in the uptake of the prevention of mother-to-child transmission of HIV recommendations before and after Option B+ implementation

PMTCT step	Description	Pre-Option B+ period	Post-Option B+ period	p-value
		01/01/2010 – 31/12/2011	**01/04/2014 - 31/03/2015**	
Assessment of HIV status in antenatal care	Number of women attended at the ANC	4027	1579	
	Pregnant women with HIV positive status known, n/N (%)	44/4027 (1.1)	7/1579 (0.4)	0.0213
	Pregnant women offered an HIV test, n/N (%)	3983/3983 (100)	1548/1572 (98.5)	0.0107
	Pregnant women accepting the HIV test, n/N (%)	3606/3983 (90.5)	1456/1548 (94.1)	<0.0001
	Pregnant women tested HIV positive in the ANC, n/N (%)	207/3606 (5.7)	39/1456 (2.7)	<0.0001
	Prevalence of HIV among pregnant women attended at the ANC, n/N	251/3650 (6.9)	46/1463 (3.1)	<0.0001
		01/01/2010 – 31/12/2011	**01/04/2014 - 31/03/2015**	
Enrolment in HIV care	Women newly diagnosed at ANC and enrolled, n/N (%)	53/207 (25.6)	35/39 (89.7)	<0.0001
		20/03/2011 - 03/05/2011	**01/04/2014 - 31/03/2015**	
PMTCT at labour ward	Number of deliveries	570	5727	
	Women with HIV positive status known at admission, n/N (%)	28/570 (4.9)	168/5727 (2.9)	0.01
	Women with unknown HIV status at admission, n/N (%)	82/570 (14.4)	1155/5727 (20.2)	<0.0001
	Women tested for HIV during labour & delivery, n/N (%)	2/82 (2.4)	345/1155 (29.9)	<0.0001
	Women tested HIV-positive during labour & delivery, n/N (%)	1/2 (50)	18/345 (5.2)	
	Prevalence of HIV among women attended at labour ward, n/N (%)	29/486 (6.0)	186/4917 (3.8)	0.0188
	HIV-exposed newborns receiving the recommended postnatal prophylaxis, n/N (%)	1/29 (3.5)	154/184 (83.7)	<0.0001

PMTCT: Prevention of mother-to-child transmission; ANC: antenatal care clinic

Pregnant women receiving HIV care at the One Stop Clinic

During the study period, 124 HIV-infected pregnant women attended the One Stop Clinic. Forty-five of them had been newly diagnosed with HIV during the current pregnancy, and 79 were under HIV care when pregnancy was reported.

Newly diagnosed HIV-infected pregnant women

Of the 39 pregnant women newly diagnosed in the ANC, 35 (90%) were enrolled into HIV care, a significant increase from the period 01/2010-12/2011 (90% versus 26%, p<0.0001) (Table 1). Another ten women were newly diagnosed and enrolled in HIV care during the study period: 7/10 from the obstetric ward and 3/10 from the voluntary counselling and testing unit. The clinical characteristics and pregnancy outcomes of these 45 women are summarized in Table 2.

Median age at enrolment was 28.4 years (interquartile range (IQR) 22.2-31.8), median CD4 counts 334 cells/µL (IQR 166–509), and 89% presented with WHO stage 1/2. Seventeen women (38%) were aware of their male partner HIV serostatus: 7/45 (16%) reported their male partner was HIV-positive and 10/45 (22%) HIV-negative. Thirty-seven women (82%) initiated ART after a median of 3 days (IQR 0–7). After a median follow-up time of 17.2 months (IQR 14.8–21.2), 28/45 (63%) women were under active follow-up at the One Stop Clinic, 4/45 (9%) had been transferred to another facility, 1/45 (2%) had died and 12/45 (27%) were lost to follow-up. Pregnancy outcomes were recorded for 35/45 women: 6/35 (17%) had an abortion, miscarriage or neonatal death, and the remainder delivered alive babies.

Women under HIV care who became pregnant

Seventy-nine women under HIV care became pregnant during the study period. The median time since enrolment was 55 months (IQR 15–74). At the time of HIV diagnosis, their median CD4 counts were 265 cells/µL (IQR 134-495) and 79% presented WHO stage 1 or 2 (Table 2). When pregnancy was reported, 60% (47/79) were aware of their male partner's serostatus: 39% (31/79) reported their male partner was HIV-positive and 20% (16/70) HIV-negative. Most women (86%, 68/79) had started ART before pregnancy. The remaining 11 women initiated ART after a median of 2 days of their pregnancy report (IQR 0–5). After a median follow-up of 19.1 months (IQR 14.5–21.8), 81% (64/79) women were under active follow-up at the One Stop Clinic, 9% (7/79) had been transferred to another facility, 4% (3/79) had died and 6% (5/79) were lost to follow-up. Pregnancy outcomes were recorded for 95% (75/79) of women: 12% (9/75) had an abortion, miscarriage or neonatal death and the rest delivered alive babies (Table 2).

Table 2: Characteristics of HIV-infected women enrolled in HIV care during pregnancy and women enrolled before pregnancy

Characteristics	New HIV diagnosis during pregnancy (N = 45)	Women under HIV care who became pregnant during follow-up (N = 79)	p-value
Time since enrolment (months), median (IQR)	-	55 (15 - 74)	
Age at pregnant report (years), median (IQR)	28.4 (22.2 - 31.8)	34 (29 - 36)	<0.0001
CD4 count at enrolment (cells/µL), median (IQR)	334 (166 - 509)	265 (134 - 495)	0.46
WHO clinical stage at enrolment, n (%)			0.05
Stage 1/2	40 (88.9)	59 (78.7)	
Stage 3/4	5 (11.1)	16 (21.3)	
Partner HIV serostatus at pregnancy report, n (%)			0.02
Positive	7 (15.6)	31 (39.2)	
Negative	10 (22.2)	16 (20.3)	
Not tested / Unknown	21 (46.7)	19 (24.1)	
Not applicable [a]	7 (15.6)	13 (16.5)	
ART status at pregnancy report			
Not on ART, n (%)	-	11 (13.9)	
On ART, n (%)	-	68 (86.1)	
Time on ART at pregnancy report (months), median (IQR)	-	45.3 (16.3 - 72.1)	
ART regimen at pregnancy report, n (%)			
NVP-based regimen	-	19 (27.9)	
EFV-based regimen	-	41 (60.3)	
PI-based regimen	-	8 (11.8)	
ART-naïve pregnant women initiated on ART, n/N (%)	37/45 (82.2)	11/11 (100)	
Time to ART initiation after pregnancy report (days), median (IQR) [b]	3 (0 - 7)	2 (0 - 5)	
Follow up time at time of analysis (months), median (IQR)	17.2 (14.8 - 21.2)	19.1 (14.5 - 21.8)	
Retention in care at time of analysis, n (%)			0.02
Active follow up	28 (62.2)	64 (81.0)	
Died	1 (2.2)	3 (3.8)	
Lost to follow-up	12 (26.7)	5 (6.3)	
Transfer to another clinic	4 (8.9)	7 (8.9)	
Pregnancy outcome [c], n (%)			0.05
Live born singleton	27 (77.1)	64 (85.3)	
Live born twins	2 (5.7)	2 (2.7)	
Abortion, miscarriage or neonatal death	6 (17.1)	9 (12.0)	

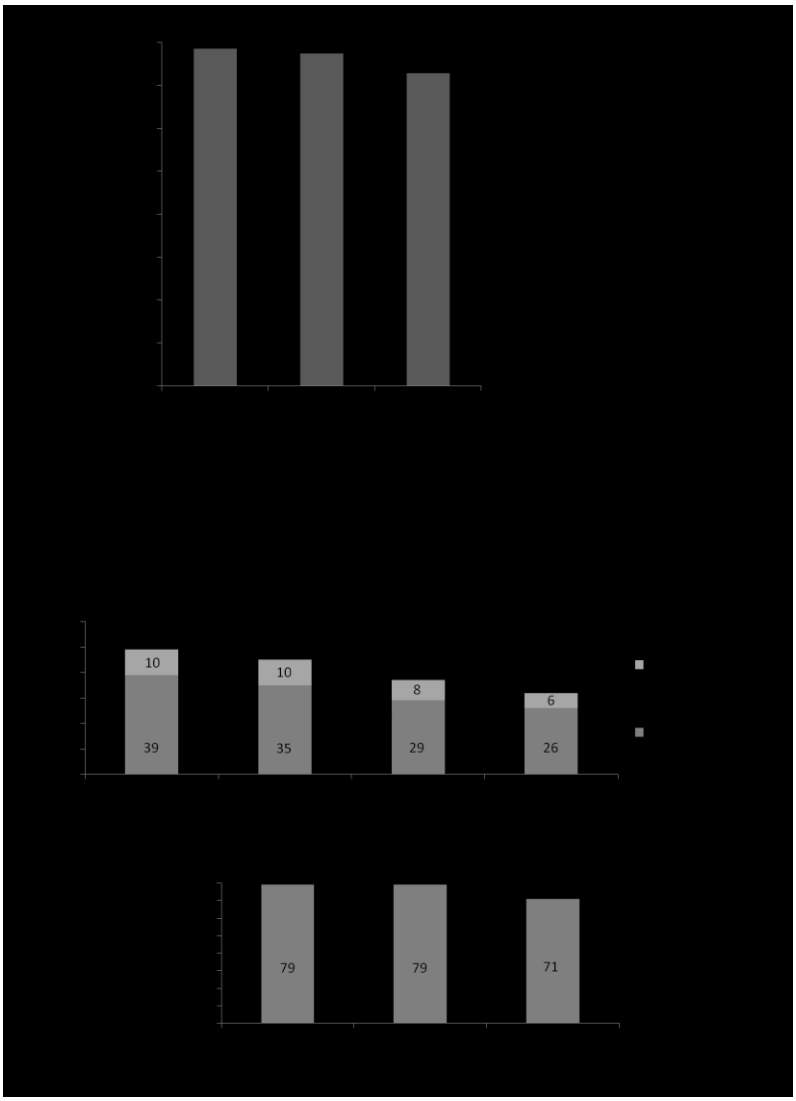
[a] Non applicable refers to women who denied having a male partner
[b] Applies to 37/45 (N=37) of the newly diagnosed women and to women under HIV care who were not on ART at pregnancy report (N = 11)
[c] For 10 newly diagnosed women and 4 women under HIV care at pregnancy report, pregnancy outcome was nor recorded (they died, were lost to follow-up or were transferred to another clinic before delivery)

IQR: interquartile range; WHO: World Health Organization; ART: antiretroviral treatment; NVP: nevirapine; EFV: efavirenz; PI: protease inhibitor.

Figure 3 shows the PMTCT cascade for newly diagnosed women (3A) and women becoming pregnant while under HIV care (3B). Compared to newly diagnosed women, those who were under HIV care at pregnancy report were older (34 versus 28.4 years, p<0.0001), had similar

CD4 counts at HIV diagnosis (265 versus 334, p=0.46), higher proportion of WHO clinical stage 3/4 (21% versus 11%, p=0.05), better knowledge of their male partner serostatus (60% versus 38%, p=0.02), lower lost to follow-up rate (6% versus 27%, p=0.02), and better pregnancy outcomes (proportion of live born babies 88% versus 83%, p=0.05) (Table 2).

Figure 3: Retention through the PMTCT cascade for women newly diagnosed with HIV during the current pregnancy (3A) and women who became pregnant while being under HIV care (3B)



* Women known to be HIV-infected (n=7) have not been included
** Women retained in care at the One Stop Clinic or transferred to another facility were considered to be under active follow-up

PMTCT: prevention of mother-to-child transmission of HIV; ANC: antenatal care clinic; ART: antiretroviral treatment; VCT: voluntary counselling and testing

PMTCT at the labour ward

The labour ward at SFRH attends women from several ANCs and receives the referrals from lower level facilities. During the study period 5,727 women delivered at the hospital. At admission, 80% (4,572/5,727) had their HIV status documented (168 HIV-positive) and for 20%

(1,155/5,727) it was unknown. Thirty percent (345/1,155) of women in this latest group were tested for HIV during labour, 5.2% (18/345) were HIV-infected. Overall, 3.8% (186/4,917) of women with known HIV serostatus giving birth at SFRH were HIV-infected. None of the women with a documented HIV-negative test during pregnancy was re-tested during labour. The comparison of the uptake of recommendations between our previous assessment and the current period is presented in Table 1. During the post-B+ period the number of women presenting at labour with unknown HIV status increased (20% versus 14%, p<0.0001), but the HIV testing rate of these women increased (30% versus 2.4%, p<0.0001). Similarly to the ANC, the HIV prevalence among pregnant women attended at the labour ward was lower than in 2010-11 (3.8% versus 6.0%, p=0.02).

HIV-exposed infants and mother-to-child transmission

During the study period, 135 HIV-exposed infants born from mothers diagnosed with HIV before pregnancy or during pregnancy or delivery were enrolled at the One Stop Clinic (Table 3). Sixty-two percent (83/135) of the mothers were diagnosed before pregnancy. Eighty eight percent (119/135) of the mothers received correct drugs for PMTCT and 75% (101/135) of the infants got the recommended postnatal prophylaxis. Median age at the time of the first HIV test was 6 weeks (IQR 5-11). By the time of analysis 2.2% (3/135) infants were HIV-infected (all diagnosed at first HIV test), 66.7% (90/135) were uninfected, and 31.1% (42/135) had a first negative HIV test but were still breastfeeding when last visited. Remarkably, none of the followed infants with an initial negative test seroconverted during the study period. Fifteen months after the last infant included was enrolled, the lost to follow-up rate was 14% (19/135) and 6% (8/136) infants had died (2 were HIV-infected).

Table 3: Characteristics of the 135 HIV-exposed infants born from mothers diagnosed before pregnancy or during pregnancy, labour and delivery

Characteristic	
Female gender, n (%)	63 (46.7)
Time of mother's HIV diagnosis, n (%)	
Before pregnancy	83 (61.5)
During pregnancy/delivery	52 (38.5)
Correctness of mother's ART for PMTCT, n (%)	
Correct	119 (88.2)
Incorrect	16 (11.9)
Correctness of infant's postnatal ARV prophylaxis, n (%)	
Correct	101 (74.8)
Incorrect	34 (25.2)
Age at first HIV test (weeks), median (IQR)	6 (5 - 11)
Timely initiation of co-trimoxazole prophylaxis, n (%) [a]	108 (84.4)
Infant feeding during the first 6 months of life, n (%)	
Exclusive breastfeeding	123 (91.1)
Replacement feeding	4 (3.0)
Animal's milk	2 (1.5)
Mixed feeding	6 (4.4)
Final serostatus of children, n (%)	
HIV negative	90 (66.7)
HIV positive	3 (2.2)
Not yet known [b]	42 (31.1)
Retention in care at time of analysis, n (%)	
Active follow up	103 (76.3)
Lost to follow-up	19 (14.1)
Transferred to another clinic	5 (3.7)
Died [c]	8 (5.9)
HIV-positive	2

[a] For 7 infants the correctness of CPT initiation was not applicable, since they were enrolled after the age of 6 weeks
[b] 19/42 infants were lost to follow-up after a first negative HIV test; 23/42 infants had a first negative HIV test but were still breastfeeding at the time of analysis
[c] Causes of death: 1 septicaemia (HIV-infected); 1 acute respiratory failure (HIV-infected); 1 congenital hydrocephaly; 1 spina bifida; 1 Kwashiorkor malnutrition (and suspected tuberculosis); 1 bacterial pneumonia; 2 unknown.

ART: antiretroviral treatment; PMTCT: prevention of mother-to-child transmission; ARV: antiretroviral; IQR: interquartile range.

During the same period 24 HIV-exposed infants born from mothers who were diagnosed after delivery were enrolled. Most of these mothers had attended an ANC during pregnancy but were not offered an HIV test. The MTCT rate in this group was 46% (11/24). Most of these infants and their mothers were identified in the hospital wards, when admitted with malnutrition, advanced HIV disease or opportunistic infections.

Discussion

This is the first study to evaluate the Option B+ cascade in Tanzania. Option B+ delivered through the One Stop Clinic model dramatically improved linkage to HIV care after diagnosis in the ANC and resulted in over 90% of enrolled women receiving ART. Retention throughout the

PMTCT pathway was challenging for newly diagnosed HIV-infected pregnant women. The observed MTCT rate (2.2%) was below the national average (9%) and the 5% threshold established for elimination of MTCT of HIV in breastfeeding populations (WHO, 2014a). Nevertheless, gaps such as the poor uptake of HIV testing in the labour ward and an almost inexistent HIV re-screening during late pregnancy remained and need to be urgently addressed.

Improvement in enrolment to HIV care. The rate of HIV testing in the ANC was > 90% both in the pre-B+ and the post-B+ periods. This is reassuring, since failure to detect HIV-infected mothers in the ANC is estimated to be responsible for 54% of paediatric HIV infections in resource-limited settings (Centers for Disease Control and Prevention (CDC), 2013). Enrolment to HIV care from ANC increased from 26% during January 2010 to December 2011 to 90% during April 2014 to March 2015. In two large urban centres in Malawi, the enrolment to PMTCT/HIV care from the ANC increased from 61% in the pre-B+ period to 87% in the post-B+ period (Kim *et al.*, 2015). However, a study from Uganda found that after Option B+ implementation, only 25% of women diagnosed in rural ANC settings were linked to HIV care. Health workers interviewed in that study suggested improving competence in HIV counselling and integration of PMTCT and chronic HIV care within the routine reproductive and child care as potential solutions to poor linkage (Mugasha *et al.*, 2014). Thus, we attribute the improved linkage to HIV care, not to Option B+ itself, but to the integration of antenatal and HIV services within the same clinic and the specialised counselling delivered through the One Stop Clinic.

The main challenge: retention through the PMTCT cascade. In our study, Option B+ was challenged by the losses of newly diagnosed HIV-infected women along the PMTCT cascade. As seen in other settings (Kim *et al.*, 2015), a substantial proportion of newly enrolled women (8/45, 18%), were lost to follow-up before ART initiation. High rates of early attrition are also seen in places where same day diagnosis and treatment initiation is the standard of care (van Lettow *et al.*, 2014; Chan *et al.*, 2016). These findings indicate that women may need time to adjust to the HIV diagnosis and understand the benefits of lifelong ART. After ART initiation, recently diagnosed women continued to drop from care, although at a lower rate: 86.5% (32/37) were retained after a median of 17.2 months. This result is better than the one reported in a recent publication from Malawi, where retention after ART initiation in the context of Option B+ was 68.5% at 12 months, 61% at 24 months and 56.3% at 36 months (Haas *et al.*, 2016). While the size of our study population was small, our better results suggest that the unhurried start of ART and continued counselling combined with joint visits for maternal care, PMTCT/HIV care and infant growth monitoring and immunization may be

responsible for the lower attrition. The better retention of women becoming pregnant while being under HIV care must be capitalized and peer-mother programs should be common in all settings (Tenthani *et al.*, 2012; Shroufi *et al.*, 2013). Importantly, the uniqueness of Option B+ challenges may be coming to an end, since many countries are implementing the "test and treat" strategy for all HIV-infected individuals (WHO, 2016). Thus, lessons learned from the implementation of Option B+ should serve to enhance not only PMTCT but also ART programs in sub-Saharan Africa.

The elimination of new paediatric HIV infections: a met target. The MTCT rate observed at the One Stop Clinic was 2.2%, achieving the target of <5% established for populations in which breastfeeding is common (WHO, 2014a). Therefore, unlike previously reported (Wudineh and Damtew, 2016), the virtual elimination of new paediatric HIV infections seems feasible in a rural setting under programmatic circumstances. The lost to follow-up rate of infants after a minimum of 15 months since enrolment was 14%, much lower than the average 34% reported for sub-Saharan African settings at only 3 months post-delivery (Sibanda *et al.*, 2013). We believe that the integrated service delivery model combined with continued counselling after delivery are responsible for these results. In line with our findings, a cluster-randomized clinical trial conducted in Nigeria showed that integration of mother and infant services resulted in better provision of PMTCT and a significant increase in retention (Aliyu *et al.*, 2016).

This success is shadowed by the infants identified as HIV-exposed after delivery. In most cases, these infants were enrolled after being admitted with symptoms and a high proportion was HIV-infected. This finding provides evidence that some mothers are still not captured by PMTCT programs. Thus, in parallel to Option B+ efforts, case-finding approaches are necessary to timely diagnose HIV-infected children outside the PMTCT pathway (Ahmed *et al.*, 2013).

Testing gaps. Twenty percent of women attended the labour ward with unknown HIV serostatus. Since over 95% of women in the region attend at least one antenatal visit (Tanzanian NBS, 2011), we attribute this finding to test shortages in some district health facilities. Intrapartum HIV testing is crucial for maximizing PMTCT programs, but, worryingly, only a third of women with unknown serostatus were tested during labour. Reasons for the low HIV testing at the labour ward are the substantial workload of the health workers and the frequent staff turnover, which hinder the efficacy of the PMTCT training and messages delivered to midwifes and nurses working there. Furthermore, HIV re-screening during late pregnancy was anecdotic in our setting. A second HIV test during the last trimester of pregnancy is recommended based on its cost-effectiveness (Soorapanth *et al.*, 2006) and the

increased risk of HIV acquisition during pregnancy (Gray *et al.*, 2005). In our hospital, 2% of women re-tested for HIV in late pregnancy had seroconverted. With this seroconversion rate, a total of 28 women may have been silently infected during pregnancy. Having re-screened all women would have resulted in a 72% increase in the number of women diagnosed in the ANC and an opportunity to prevent their infants to become infected. Re-screening and intrapartum testing can act as safety nets to ensure the success of PMTCT. Continuous education of the attending staff and a strengthen supply chain of tests are crucial to bridge these testing gaps.

This study has some limitations. First, we did not include mother-infant pairs, but pregnant women and HIV-exposed infants enrolled during the study period. The number of infants enrolled in a year exceeded the number of women. This is due to yearly variations and transferred and orphan infants, but also to some mothers from peripheral facilities preferring their infants to receive comprehensive care at the One Stop Clinic. Still, a significant proportion of the infants were born from mothers enrolled during the study, and the uptake of the guidelines could be satisfactorily assessed. Second, an important part of women delivering at SFRH did not attend the local ANC, and complete antenatal data was not available for them. Third, it is possible that some women ascertained as lost to follow-up self-transferred to another facility, as it is common in district high level facilities as ours (Koole *et al.*, 2014; Wilkinson *et al.*, 2015). Finally, since 14% of the HIV-exposed infants were lost to follow-up while breastfeeding, it is possible that the MTCT rate of 2.2% underestimates the true transmission rate. However, even presuming a 15% transmission through the breast milk among the lost to follow-up infants, the cohort MTCT rate would still be below 5%.

The strength of this study is being the first one to comprehensively analyse the Option B+ cascade in a Tanzanian setting, including all the steps of the PMTCT pathway and the long follow-up time for both mothers and infants.

Conclusions

In summary, Option B+ was successfully implemented in this rural African setting through an integrated service delivery model. Most diagnosed women were linked into HIV care, received appropriate ART and the MTCT rate was below 5%. However, important testing gaps that may have left women undiagnosed were observed. The One Stop Clinic is a feasible, inexpensive and scalable Option B+ delivery model that could be extrapolated to similar rural settings. Despite the success, caution is warranted and additional strategies to ensure universal HIV testing for pregnant and delivering women and to improve early ART retention of newly diagnosed women are crucially needed.

Acknowledgements

Other members of the KIULARCO Study Group are: Aschola Asantiel, Adolphina Chale, Diana Faini, Ingrid Felger, Gideon Francis, Hansjakob Furrer, Tracy R Glass, Speciosa Hwaya, Bryson Kasuga, Andrew Katende, Namvua Kimera, Yassin Kisunga, Thomas Klimkait, Antonia Luhombero, Ezekiel Luoga, Hery Mapesi, Leticia Mbwile, Mengi Mkulila, Julius Mkumbo, Margareth Mkusa, Dorcas K Mnzava, Germana Mossad, Dolores Mpundunga, Daimon Msami, Athumani Mtandanguo, Selerine Myeya, Sanula Nahota, Regina Ndaki, Agatha Ngulukila, George Sikalengo, Fiona Vanobberghen, and Maja Weisser.

The authors are grateful to all the pregnant women, mothers and families attended at the One Stop Clinic and the staff from the Saint Francis Referral Hospital in Ifakara.

8. *Study 4*: A Case Series of Acquired Drug Resistance-Associated Mutations in HIV-infected Children: an Emerging Public Health Concern in Rural Africa

Open Forum Infect Dis. 2015 Dec 17;3(1):ofv199

A Case Series of Acquired Drug Resistance-Associated Mutations in HIV-infected Children: an Emerging Public Health Concern in Rural Africa

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Abstract

The acquisition of drug-resistance mutations among African children living with HIV on antiretroviral treatment has been scarcely reported. This threatens the overall success of antiretroviral programs and the clinical outcomes of children in care. We present a well-characterized series of children from rural Tanzania with acquired drug-resistance mutations to contribute to the better understanding of this emerging public health concern.

Introduction

There are few data on the acquisition of drug-resistance mutations among African children living with HIV on antiretroviral treatment (ART). Overall, in resource-limited settings, HIV-1 treatment failure in children is estimated to be 40% (Sigaloff *et al.*, 2011). Virologic suppression and long-term treatment success are harder to achieve than in adults(Sutcliffe *et al.*, 2008). This is mostly due to high pre-ART viral loads (VL), poorer virologic response, and risk of sub-therapeutic drug concentrations caused by limited paediatric drug formulations, variable pharmacokinetics, and rapid changes in body weight (Abrams *et al.*, 1998; van Rossum, Fraaij and de Groot, 2002; Walker *et al.*, 2004; Menson *et al.*, 2006; Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Study Group *et al.*, 2008; Sigaloff *et al.*, 2011). These factors, often associated with suboptimal adherence, may promote the emergence of drug-resistance mutations. Only one study from Kenya has described the pattern of acquired drug-resistance mutations in African children presenting ART failure (van Rossum, Fraaij and de Groot, 2002; Wamalwa *et al.*, 2013). In Tanzania, a small study found a virologic failure (VF) rate of 58%, 100% with drug-resistance mutations (Bratholm *et al.*, 2010).

The emergence of acquired drug-resistance mutations in children threatens ART programs in sub-Saharan Africa and needs to be studied further. We present a well-characterised series of children from a rural Tanzanian setting with treatment failure due to the acquisition of drug-resistance mutations.

Material and methods

Study setting and population

The children in this study attend the Chronic Diseases Clinic of Ifakara (CDCI), in the Saint Francis Referral Hospital. The CDCI works in cooperation with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute, and the University Hospitals of Basel and Bern. Patients attending the CDCI are offered informed consent to be enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) (Haraka *et al.*, 2015). The KIULARCO study received ethical clearance from the corresponding ethical review boards in Tanzania and Switzerland.

This is the largest peripheral HIV cohort in Tanzania with almost 8000 patients. In 01/2013 a Paediatric and Prevention of Mother-To-Child Transmission (PMTCT) unit, named "The One Stop Clinic of Ifakara", was established within the CDCI. By 03/2015, 340 children and adolescents were under active follow-up. Care and treatment for patients is provided according to the National AIDS Control Program. CD4 counts are used to routinely monitor the ART response. VL is requested by clinicians after treatment failure is suspected due to poor immunological or clinical evolution. In case VL is detectable, HIV drug-resistance genotyping is performed.

Clinical data

At each visit, clinical, laboratory and pharmacy data are collected electronically. Adherence is estimated using self-reported adherence and pill counting and considered "suboptimal" if <95% of the prescribed pills have been taken. The individual prescriptions were reviewed to assess their adequacy. For children transferred from other facilities, ART dosage was checked by direct observation of their drugs at the enrolment visit.

Viral load and genotypic resistance testing

Blood samples were collected in 8mL BD Vacutainer EDTA collection tubes. Plasma HIV RNA VL and HIV drug-resistance genotypes were determined at the Ifakara Health Institute laboratory. Cell-free plasma was collected by centrifugation at 956rcf for 5 minutes and frozen at -80^{0}C until testing for VL or drug-resistance genotyping. HIV RNA from plasma was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Viral RNA quantification was performed with the TaqMan® RNA-to-CT™ 1-Step Kit (Life Technologies) using the StepOne Real Time PCR system (Life Technologies), with a detection limit of 200 viral RNA copies/mL. HIV drug-resistance genotyping was performed by Sanger sequencing on an ABI Genetic Analyser (4-capillary model 3130) using a validated in-house PCR protocol (Masimba *et al.*, 2013).

HIV-1 drug-resistance was predicted according to the Stanford University's HIV Drug Resistance Database Program version 6.2.0 (http://hivdb.stanford.edu).

Results

We present a series of children with acquired drug-resistance mutations: 10/12 enrolled in the CDCI at HIV diagnosis and 2/12 (#11, #12) transferred to our clinic after ART initiation. Six patients (#1 - #6) were identified through a previous cross-sectional analysis within KIULARCO. The remaining six children were identified prospectively after presenting unsatisfactory evolution: patients #7, #8 and #9 presented poor CD4 increase, although they did not meet the

WHO criteria of immunological failure; patient #10 presented clinical failure; patient #11 had been exposed to low doses of antiretrovirals; and patient #12 presented with both immunological and clinical failure.

Clinical characteristics

The clinical characteristics of the patients are summarized in Table 1. Median age at ART initiation was 6.4 years (IQR 5.3, 9.4). Ten children were orphans. None was diagnosed through a PMTCT program, either had documented exposure to PMTCT. Remarkably, 10/12 children were born before 2006, year in which the first PMTCT intervention was scaled-up to our district. All children were initiated on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen. During follow-up, 7/12 had been exposed to more than one first-line regimens, mostly due to unavailability of drugs. In 3/7 cases, children were switched back and forth more than once. Only one child (#12) was treated for tuberculosis before presenting treatment failure. Median time on ART at the time when VF was detected was 3.7 years (IQR 3.2, 5.3). Most patients (11/12) presented VL >1000 copies/mL. Suboptimal adherence was common (7/12). Information about the correctness of the antiretroviral dosages prescribed was available for 10/12, and 7/10 had been exposed to sub-therapeutic doses for periods ranging from two months to three years. All prescription errors were due to failure to adjust the doses to the current weight, affecting in most cases all the regimen drugs. Only 4/12 children met the WHO criteria for clinical and/or immunological failure: patients #6 and #12 presented both immunological and clinical failure with persistent CD4 levels below 100 cell/mm^3 and severe malnutrition; patient #4 had persistent CD4 levels below 100 cell/mm^3; and patient #10 presented clinical failure due to severe malnutrition and although the CD4 evolution was unsatisfactory he did not met the WHO criteria for immunological failure.

Table 1. Clinical characteristics and antiretroviral drug resistance mutations of twelve children living with HIV in rural Tanzania

Child	Age at ART initiation (years)	Orphan status	Baseline HIV genotyping	CD4 count in cell/mm^3 (and %) at ART initiation	1st line ART regimens prescribed	Number of 1st line ART switches	History of suboptimal adherence	Under dosage of drugs	Time on ART when DRM detected (years)	VL at the time of treatment failure detection (copies/mL)	NNRTI resistance mutations	NRTI resistance mutations	2nd line regimens	VL ≥ 6 months after switched to 2nd line (copies/mL)
1	4.5	Maternal orphan	No DRM	238 (7%)	d4T/3TC/NVP	NA	Yes	Yes	4.6	5,396	A98G, K103N, V108I, K238T	M41L, V75M, M184V, L210W, T215Y	TDF/FTC/LPV/r	Not done *
2	4.9	Double orphan	No DRM	425 (13%)	d4T/3TC/NVP AZT/3TC/NVP TDF/3TC/EFV	3	No	Yes	3.4	330	Y181C	M184V	TDF/FTC/LPV/r	undetectable
3	13.2	Non orphan	No DRM	317 (10%)	AZT/3TC/EFV TDF/3TC/EFV	1	Yes	No	2.7	22,071	V90IV, K103N, P225H	Y115FY, M184MV	AZT/3TC/ATV/r	N/A †
4	6.1	Paternal orphan	No DRM	152 (12%)	d4T/3TC/NVP	NA	No	Yes	1.2	174,546	A98G, K103N, V108I, K 238T	M41L, V75M, M184V, L210W, T215Y	TDF/FTC/LPV/r	345
5	11.7	Paternal orphan	No DRM	124 (12%)	d4T/3TC/NVP AZT/3TC/NVP	1	Yes	No	3.3	1,340	K101E, Y181C, H221Y	-	TDF/FTC/ATV/r TDF/FTC/LPV/r	N/A ‡
6	11.5	Paternal orphan	No DRM	16 (1%)	AZT/3TC/EFV AZT/3TC/NVP	2	Yes	Yes	4.0	22,623	K103KN	M184IMV, L210LW	TDF/FTC/LPV/r ABC/3TC/LPV/r	124,276
7	8.7	Double orphan	Not done §	375 (12%)	AZT/3TC/EFV	NA	No	Yes	5.8	1,478	-	M184V	TDF/FTC/ATV/r TDF/FTC/LPV/r	N/A ‖
8	6.6	Maternal orphan	No DRM	331 (10%)	AZT/3TC/EFV	NA	Yes	N/A	3.3	31,000	L100I, Y188L	M184V	TDF/FTC/LPV/r	N/A ‡ ¶
9	0.8	Non orphan	Not done § #	66 (2%)	d4T/3TC/NVP AZT/3TC/NVP	2	No	No	5.1	5,648	K101E, G190A	M41L, D67N, T69L, V75M, M184V, L210W, T215Y	TDF/FTC/LPV/r	undetectable
10	5.4	Maternal orphan	Not done §	201 (3%)	d4T/3TC/NVP AZT/3TC/EFV	1	Yes	N/A	7.0	3,775	V90I, K103N, V108I, K238T	M41L, D67G, K70R, V75M, M184V, L210W, T215F, K219E	TDF/FTC/LPV/r	N/A **
11	5.8	Double orphan	N/A ††	158 (no % information)	AZT/3TC/EFV	NA	Yes	Yes	1.8	Not done *	A98AG, K101P, K103N, E138AE	M184V, T215F	TDF/FTC/LPV/r	Not done *
12	7.0	Double orphan	N/A ††	No information	AZT/3TC/EFV ABC/3TC/EFV	1	No	Yes	7.3	386,400	A98G, K103S, G190A, Y318F	M41L, D67N, L74I, M184V, L210W, T215Y	TDF/FTC/LPV/r	N/A ‖

* VL not done due to a technical problem
† The patient opted to stop ART
‡ At the time of the manuscript writing the patient had not yet completed 6 months on second-line ART
§ Pre-ART sample was not available
‖ The patient was transferred to another facility before completing 6 months on second-line ART
¶ VL was done six weeks after having been switched to 2nd line and is was 260 copies/mL
# The mother's pre-ART sample genotype did not show DRM mutations
** The patient interrupted ART three times since switched to second-line ART
†† The patient initiated ART in another facility and pre-ART sample was not available
ART: antiretroviral treatment; VL: viral load; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside/nucleotide reverse transcriptase inhibitor; d4T: stavudine; AZT: zidovudine; 3TC: lamivudine; FTC: emtricitabine; ABC: abacavir; TDF: tenofovir; NVP: nevirapine; EFV: efavirenz; LPV/r: ritonavir-boosted lopinavir; ATV/r: ritonavir-boosted atazanavir

Genotypic resistance profile

Ten children carried virus with double resistance to NNRTIs and nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). None harboured major resistance to Protease Inhibitors (PIs) (Table 1).

NRTI mutations were seen in 11/12 patients, mostly M184V (10/11). Six children harboured thymidine analogue mutations, the majority (5/6) carrying ≥2. NNRTI mutations were observed in 11/12 children, being K103N the commonest (5/11).

Pre-ART plasma samples were available for 7/10 patients enrolled at HIV diagnosis. None of them presented pre-ART drug-resistance mutations, suggesting that they developed these mutations after treatment initiation. For another patient (#9), diagnosed together with her mother after cessation of breastfeeding, a pre-ART sample from the mother did not show resistance-associated mutations. The remaining four children presented history of poor adherence and/or under-dosage of drugs, rendering the acquisition of resistance mutations likely.

Outcome

All patients were switched to a PI boosted with ritonavir (PI/r) + 2NRTIs regimen. Based on the resistance testing the regimens prescribed contained no predicted active NRTI in 5/12 cases and one predicted active NRTI in 6/12 cases. Only one patient obtained a regimen with two active NRTIs.

By the time of the analysis 6/12 children were under active follow-up and had completed six months of second-line ART with good self-reported adherence and compatible pill counting, 2/12 had not completed six months on the PI/r-based regimen, 1/12 had interrupted ART repeatedly, 2/12 had been transferred, and 1 had stopped ART (Table 1).

VL was performed in 4/6 children who had completed six months on second-line ART and 3/4 presented satisfactory VL reduction. The remaining patient, aged 17, presented a VL five-fold higher than the one when VF was detected, but HIV genotyping showed no drug-resistance mutations, suggesting low compliance with ART.

Discussion

This case series raises concern about a scarcely reported emerging public health concern in sub-Saharan Africa. To our knowledge, only one study from Kenya has described the pattern of acquired drug-resistance mutations in children failing ART in Africa (Wamalwa *et al.*, 2013). In this cohort study 34% (34/100) of patients presented VF, of whom 68% (23/34) had drug-resistance mutations, 14/23 harbouring multiclass mutations. Similar to our findings, the commonest mutations were M184V and K103N. Factors related to VF in children, such as suboptimal adherence, non-parental

caregiver, and ART regimen switches (Sigaloff *et al.*, 2011; Zoufaly *et al.*, 2013; Mutwa *et al.*, 2014) were common among our patients. Furthermore, we have identified a frequently overlooked factor: the prescription of inadequate doses of antiretrovirals. Awareness needs to be raised among health workers and tools to facilitate the prescription of paediatric formulations need to be widely disseminated and routinely used (ICAP, 2006).

Importantly, 8/12 children did not meet the WHO criteria of clinical or immunological failure (WHO, 2013) despite presenting with multi-class drug-resistance mutations, thus emphasising the urgent need for routine VL monitoring in children.

Four patients had VL monitored six months after switching. One teenager had a very high VL but no evidence of drug-resistance mutations, suggesting that both self-reported and pill-counting adherence were inaccurate. This case reminds the difficulties of some adolescents to adhere to ART. Strategies to improve disclosure of infection status and adherence need to be further developed in partnership with teenagers.

Most patients (11/12) could not receive three active second-line drugs. Recent studies show that the rates of virologic suppression after switching to a PI/r + 2NRTIs regimen remain high despite resistance to both NRTI and NNRTI (Wamalwa *et al.*, 2013; Paton *et al.*, 2014). However, these findings require further confirmation in children, who present a higher rate of acquired drug-resistance mutations and face the challenges of longer exposure to ART than adults. Yet, in most African settings, few paediatric regimens are available and advocating for child-friendly formulations is necessary. The inclusion of integrase inhibitors such as dolutegravir in paediatric ART programs in resource-limited settings needs to be explored urgently. The upcoming clinical trial PENTA 20 (www.clinicaltrials.gov) may provide key information to support its roll-out in Africa.

This study has limitations. First, it presents a small number of patients. However, given the scarcity of published data on acquired drug-resistance mutations in children in Africa, it provides a valuable insight into this public health concern. Second, we are unable to assess the prevalence of acquired drug-resistance mutations. However, preliminary results of an ongoing study in our cohort identified 25% prevalence of VF among children on ART (L Muri, personal communication). Finally, it is possible that the mutations in the pre-ART plasma samples dropped below the level of detection of the genotypic assay used and we were not able to detect them.

In conclusion, after a decade of successful rollout of ART in Africa, children and adolescents still represent an underprivileged population. ART coverage is lower and failure rates are higher than in adults. Children living with HIV have peculiarities and needs that must be acknowledged by the often

overwhelmed health workers, and specific policies targeting the paediatric population should be implemented. Moreover, VL is not available in most African treatment centres, leading invariably to a late recognition of failure and development of multi-class drug-resistance mutations. New classes of antiretrovirals and their adequate paediatric formulation are urgently needed to ensure the long-term survival of millions of children living with HIV in sub-Saharan Africa.

Funding

This work was supported by the funders of the Chronic Diseases Clinic of Ifakara and its Paediatric and PMTCT unit, the One Stop Clinic: the Ministry of Health and Social Welfare of Tanzania; the Swiss Tropical and Public Health Institute; the Ifakara Health Institute; the Government of the Canton of Basel; USAID; and the Merck for Mothers Global Giving Program.

Acknowledgement

We would like to acknowledge the collaboration of Joelle Bader, who performed the viral load and HIV genotyping of some of the samples. Moreover, the authors are grateful to the children and caregivers who are part of this case series and all the staff of the Chronic Diseases Clinic of Ifakara.

Conflict of interest

The authors have no conflict of interests to declare.

Authors' contributions

AG, LM, AN, TK and EL conceived and designed the case series. LM and AN performed the lab analysis. AG and LL provided clinical care to the children. AG, LM, AN, TK and EL drafted the manuscript. DN, LL, IF, CH, MT, MB, TK and EL reviewed the manuscript.

9. *Study 5*: Development of HIV Drug Resistance and Therapeutic Failure in Children and Adolescents in Rural Tanzania – An Emerging Public Health Concern

AIDS. 2017 Jan 2;31(1):61-70

Development of HIV Drug Resistance and Therapeutic Failure in Children and Adolescents in Rural Tanzania – An Emerging Public Health Concern

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Abstract

Objective: To investigate the prevalence and determinants of virologic failure (VF) and acquired drug resistance-associated mutations (DRM) in HIV-infected children and adolescents in rural Tanzania.

Design: Prospective cohort study with cross-sectional analysis.

Methods: All children ≤18 years attending the paediatric HIV Clinic of Ifakara and on antiretroviral treatment (ART) for ≥12 months were enrolled. Participants with VF were tested for HIV-DRM. Pre-ART samples were used to discriminate acquired and transmitted resistances. Multivariate logistic regression analysis identified factors associated with VF and the acquisition of HIV-DRM.

Results: Among 213 children on ART for a median of 4.3 years, 25.4% failed virologically. ART-associated DRM were identified in 90%, with multiclass resistances in 79%. Pre-ART data suggested that >85% had acquired key mutations during treatment. Suboptimal adherence [OR=3.90; 95%CI 1.11-13.68], female sex [OR=2.57; 95%CI 1.03-6.45], and current non-nucleoside reverse transcriptase inhibitor-based ART [OR=7.32; 95%CI 1.51-35.46 compared to protease inhibitor-based] independently increased the odds of VF. $CD4^+$ T cell percentage [OR=0.20; 0.10-0.40 per additional 10%] and older age at ART initiation [OR=0.84per additional year-of-age; 95%CI 0.73 to 0.97] were protective(also in predicting acquired HIV-DRM). At the time of VF, less than 5% of the children fulfilled the WHO criteria for immunologic failure.

Conclusion: VF rates in children and adolescents were high, with the majority of ART-failing children harbouring HIV-DRM. The WHO criteria for immunologic treatment failure yielded an unacceptably low sensitivity. Viral load monitoring is urgently needed to maintain future treatment options for the millions of African children living with HIV.

Background

In 2015, 1.8 million children were living with HIV worldwide, the vast majority in sub-Saharan Africa (SSA) (UNAIDS, 2014, 2016b). The roll-out of antiretroviral therapy (ART) in resource-limited countries has resulted in a reduction of HIV-related morbidities and mortality and an increased life expectancy of infected adults and children (Puthanakit *et al.*, 2007; Patel *et al.*, 2008). However, globally only 49% of the children in need have access to treatment (UNAIDS, 2016b). In addition to the low ART coverage in children, long-term treatment success and virologic suppression are harder to achieve in this population (van Rossum, Fraaij and de Groot, 2002), mostly due to high pre-ART viral loads and the risk of sub-therapeutic drug concentrations caused by limited paediatric drug formulations, variable pharmacokinetics, and continuous bodyweight changes (Abrams *et al.*, 1998; Menson *et al.*, 2006; Sigaloff *et al.*, 2011).

The lack of reliable HIV rapid tests for infants and the limited treatment monitoring in most resource-limited settings, often combined with advanced immunosuppression at ART initiation, further aggravate treatment outcomes (Sutcliffe *et al.*, 2008; Bratholm *et al.*, 2010). Together with the challenge of adherence during childhood and adolescence, these factors promote the emergence of HIV drug-resistance mutations (HIV-DRM) (Simoni *et al.*, 2007; Sigaloff *et al.*, 2011). Moreover, although multiple studies confirm that immunological and clinical criteria fail to timely detect treatment failure among children and adolescents (Kantor *et al.*, 2009; van Oosterhout *et al.*, 2009; Mutwa *et al.*, 2014), most resource-limited settings do not have plasma HIV RNA viral load (VL) monitoring available (WHO, 2010b). Given the limited ART options in SSA, the emergence of newly acquired HIV-DRM in children is likely to lead to poor clinical outcomes including a reduced survival.

Two recent systematic reviews on the effectiveness of ART among children found virologic success rates of 40% to 81% after twelve months on treatment (Sutcliffe *et al.*, 2008; Ciaranello *et al.*, 2009). Recently, not yet published data collected by the Tanzanian CDC comparing fifteen different settings across the country demonstrated a high average virologic failure (VF) rate of 38.8% among children, with HIV-DRM found in 84.4% of the failing children.

We assessed the prevalence and determinants of VF and acquired HIV-DRM after long-term ART exposure within a large paediatric HIV cohort in rural Tanzania.

Patients and methods

Study site and population

All data were prospectively collected from participants enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) after getting informed consent from the patient or caregiver if younger than 19 years. This ongoing, open, prospective cohort is comprised of all patients enrolled at

the Chronic Diseases Clinic of Ifakara, which serves as a Care and Treatment Centre for HIV/AIDS patients within the Saint Francis Referral Hospital. This is the largest health care facility in the Kilombero district, in southern Tanzania, providing treatment and care for a population of approximately 600,000 inhabitants and estimated 38,000 people living with HIV. Established in 2004, this was the first rural clinic accredited to be a Care and Treatment Centre of the National AIDS Control Program in the country, and over 9000 patients have been enrolled into care. Since 2013 the Chronic Diseases Clinic has a family-centred unit named the "One Stop Clinic of Ifakara", where care to HIV-infected children, mothers and their families is provided by a specially trained team (Gamell, A et al., 2015a, 2015b).

At each clinical visit, comprehensive clinical data is systematically collected through electronic medical records. Blood samples are drawn at routine clinic visits before ART initiation, two weeks, 3 months and every 6 months thereafter. Plasma is cryopreserved at -80ºC (Mossdorf *et al.*, 2011; Vanobberghen, F et al., 2015a, 2015b).

All HIV-infected children and adolescents aged ≤18 years enrolled in KIULARCO and who had been on ART for at least twelve months were included in this study.

Viral load testing and HIV genotyping

Blood samples were collected in 8mL BD Vacutainer EDTA collection tubes. Cell-free plasma was collected by centrifugation at 956 g for 5 minutes and frozen at -80^{0}C until testing for HIV RNA VL or viral drug-resistance. Assays for VL and sequencing for HIV drug-resistance were performed at the Ifakara Health Institute laboratory inIfakara.HIV RNA from 400µL plasma was extracted using the NucleoSpin® Virus kit (Macherey-Nagel, Oensingen, Switzerland) according to the manufacturer's protocol. Viral RNA quantification was performed using a validated in-house protocol [23]with the Brilliant III Ultra-Fast QRT-PCR Master Mix (Agilent Technologies, La Jolla CA, USA) using the StepOne™ Real-Time PCR System (Applied Biosystems, Foster City CA, USA), with a detection limit of 200 viral RNA copies/mL of plasma. HIV drug-resistance genotyping was performed by Sanger sequencing on a 3130 Genetic Analyser 4-capillary model (Applied Biosystems, Foster City CA, USA) using a validated in-house PCR protocol (Masimba *et al.*, 2013).

Statistical analysis

The primary outcomes were VF, defined as a viral RNA level of ≥1000 copies/mL after at least twelve months on ART, and the acquisition of major HIV-1 DRM in failing patients. For data description, the numeric variables were displayed with medians and interquartile ranges (IQRs) whereas the categorical variables were presented in proportions. Associations between considered variables and

VF and HIV-DRM were assessed using multivariate logistic regression models. All analyses were performed using STATA, version 14 (Stata Corporation, College Station, TX, USA).

Ethical approval

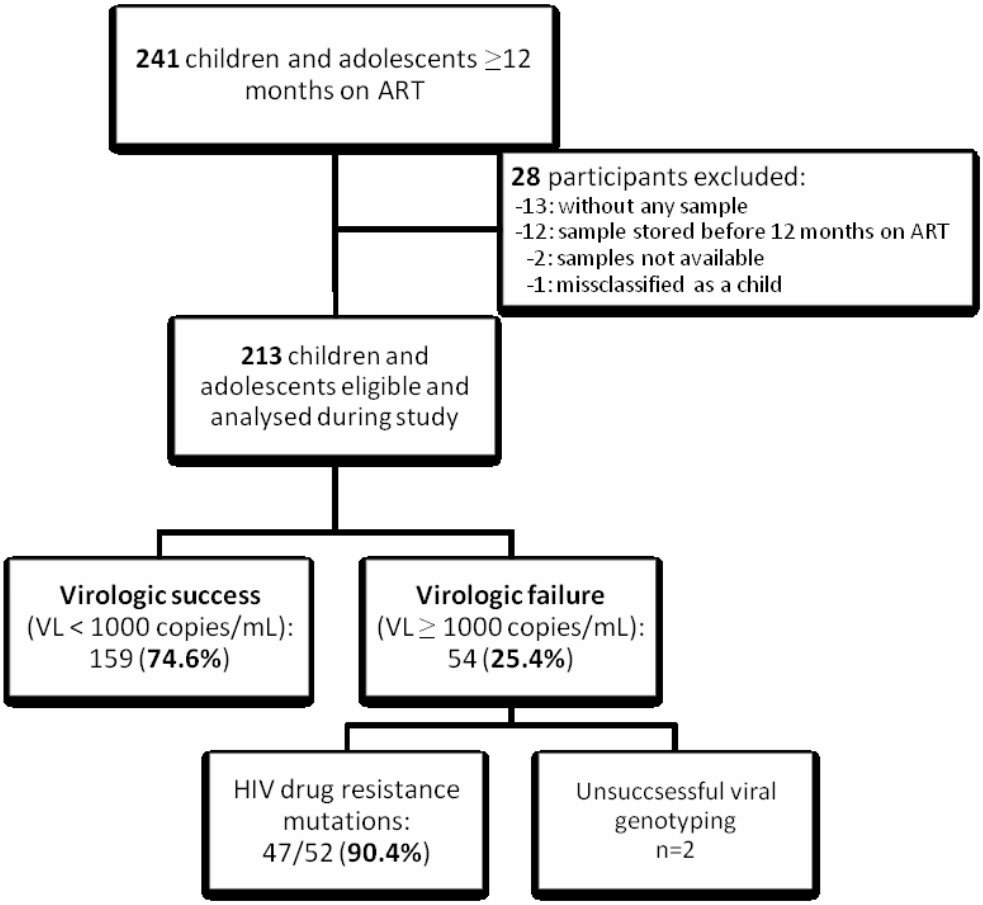
The KIULARCO study received ethical approval from the Ifakara Health Institute Institutional Review Board, the National Institute for Medical Research of Tanzania, the Tanzanian Commission of Science and Technology, and the Ethics Committee of the University and State of Basel.

Results

Characteristics of study population

At the time of analysis, 241 children and adolescents had been on ART for longer than 12 months. Twenty-eight patients were excluded due to several causes (Figure 1). The remaining 213 children contributed 902.2 person-years of follow-up. The characteristics of the study participants are described in Table 1. The median age was eleven years (IQR 7.5 – 14.4) and 43% were female. Fifty-five per cent were classified as WHO clinical stage 3 or 4, the median CD4 percentage was 12.2% (IQR 6.3-19.3) and 12.4% reported prior antiretroviral exposure at the time of enrolment in the cohort.

Figure 1. Profile of the paediatric study cohort at the Chronic Disease Clinic Ifakara in Ifakara, Morogoro, Tanzania, with virologic outcomes and the presence of drug resistance mutations



Initial regimen consisted mostly of co-formulated zidovudine (AZT)/ lamivudine(3TC) with a non-nucleoside reverse transcriptase inhibitor (NNRTI) in 54.9% children, whereas stavudine (d4T) / 3TC with nevirapine (NVP) was used in39% of the participants. At the time of investigation, the median time on ART was 4.45 years (IQR 2.4 – 6.0), 84% were on an NNRTI-based regimen and 15% (32/213) on a boosted protease inhibitor (PI)-based regimen, 28 of them as a second-line treatment. The decision to switch to a second-line regimen had been based on clinical and immunological criteria, as VL monitoring had not been available for routine monitoring of treatment success. Eighty-six percent reported good adherence to ART (defined as no missed doses during the last four weeks) and 23.5% had experienced ART regimen changes due to drugs unavailability or shortage, with a median of three regimen switches (range 0-16). Ten children (4.7%) were reported to be lost to follow-up since the last clinical visit.

Table 1. Characteristics of children and adolescents enrolled in the Kilombero and Ulanga Antiretroviral Cohort that had been on antiretroviral treatment for at least 12 months.

Characteristics	n	Overall cohort (N=213)	Virologic success (n=159)	Virologic failure (n=54)
Female (%)	213	92 (43.2)	65 (40.9)	27 (50)
Age, years (IQR)	213	11.0 (7.5-14.4)	11.2 (7.6-13.8)	10.3 (7.2-15.2)
Age at ART initiation, years (IQR)	206	6.4 (3.1-9.5)	6.3 (3.9-9.3)	7.2 (2.5-9.7)
Time on ART, years (IQR)	209	4.45 (2.4-6.0)	4.5 (2.4-6.1)	4.1 (2.5-5.6)
Any prior ART exposure (%)	193	24 (12.4)	19 (13.1)	5 (10.4)
Current CD4 count in cells/µL, median (IQR)	176	636 (440-901)	680 (477-911)	485 (364-881)
CD4 count in cells/µL at pre-ART, median (IQR)	139	295 (123-561)	292 (134-523)	318 (78-643)
Current CD4 percent, median (IQR)	171	29 (23-34.1)	30.2 (24.8-35.9)	24.4 (15-29)
CD4 percent at pre-ART, median (IQR)	137	12.2 (6.3-19.3)	13 (7-18.6)	9.8 (2.7-21.5)
Current WHO clinical stage 3-4 (%)	209	141 (67.5)	104 (67.1)	37 (68.5)
WHO clinical stage 3-4 at pre-ART (%)	186	102 (54.8)	79 (57.3)	23 (57.9)
Distance to CTC (km)	183	1 (1-25)	1 (1-25)	1 (1-33)
Good adherence[1] (%)	213	184 (86.4)	142 (89.3)	42 (77.8)
Lost to follow-up since last visit[2] (%)	213	10 (4.7)	7 (4.4)	3 (5.6)
Initial ART regimen (%)	195			
d4T+3TC+NVP		76 (39)	58 (39.5)	18 (37.5)
AZT+3TC+NVP		37 (19)	24 (16.3)	13 (27.1)
AZT+3TC+EFV		70 (36)	56 (38.1)	14 (29.2)
Others		12 (6)	9 (6.1)	3 (6.2)
Current ART regimen (%)	213			
PI-based regimen		32 (15.0)	24 (15.1)	8 (14.8)
NNRTI-based regimen		179 (84.0)	135 (84.9)	44 (81.5)
Others		2 (1.0)	0 (0)	2 (3.7)
Number of regimen switches (IQR)	213	3 (2-5)	3 (2-5)	4 (2-5)
ART change due to stock-out (%)	213	50 (23.5)	36 (22.6)	14 (25.9)
BMI for age z-score (IQR)	209	-0,74 (-1,42 to -0,03)	-0,82 (-1,5 to -0,12)	-0,57 (-1,39 to 0,16)
Weight for height z-score[3] (IQR)	14	0,21 (0,01 to 1,08)	0,14 (0,1 to 0,76) (n=9)	0,71 (0,08 to 1,1) (n=5)

[1]Defined as any missed dose during the last four weeks, reported by the patient or their caregiver
[2]Last visit more than 6 months + 60 days
[3]Includes only children < 5 years (n=14)
IQR: interquartile range; ART: antiretroviral treatment; CTC: care and treatment centre; d4T: stavudine; 3TC: lamivudine; NVP: nevirapine; AZT: zidovudine; EFV: efavirenz, PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; BMI: body mass index.

Virologic outcome

At the time of investigation, 159/213 (74.6%) participants had viral plasma levels below the WHO threshold for VF (<1000 copies/mL). In total, 92/213 children and adolescents (43.2%, 95% CI 36.5 – 49.8) had detectable HIV-1 in plasma. Fifty-four children (25.4%, 95% CI 19.5 - 31.2) had VF with a median VL of 20615 copies/mL, and 38 (17.8%, 95% CI 12.7 – 23.0) had detectable viral plasma levels below the WHO threshold: 26 (12.2%) <500 copies/mL, and 12 (5.6%) between 500 and 1000

copies/mL. Forty-six of the 54 patients with VF were on a first-line ART regimen and eight on second-line treatment. Patients presenting VF were switched to a new ART regimen according to the genotype produced during this study.

Immunologic response after ART initiation and virologic outcome

The average number of $CD4^+$ T cell percentage (CD4%) measurements from the 213 participants was six (range 1-16). The median CD4% rose from 12.2%at ART initiation to 26%after twelve months on treatment, reaching a maximum of 30.5% after 60 to 66 months on ART. By comparing the CD4% recovery of patients with and without VF (Figure2), the immune cell recovery in those with VF tended to be diminished, reaching only 22%after 12 months, compared to 27.5%among patients without VF. This difference, however, did not reach statistical significance. Although there was a trend for lower CD4% among participants with VF, they also showed a median CD4% recovery after ART initiation, which remained stable at or above 20%. In this group, the median CD4% after treatment initiation never dropped below the threshold of 10%, a criteria of immunological failure in children below five years (WHO, 2013), and the median $CD4^+$ T cell count stabilised above 500 cells/µL.

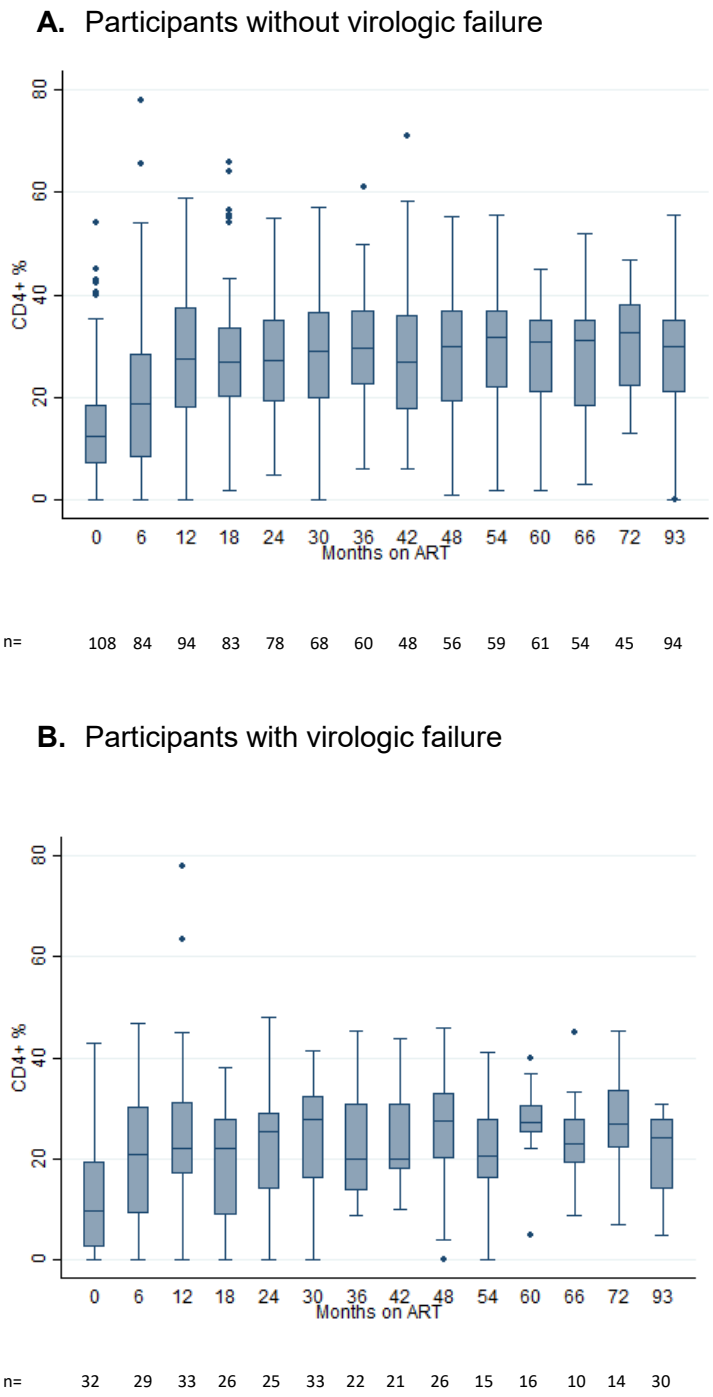


Figure 2. $CD4^+$ T cell percentage recovery among the 213 children analysed within this study.

The horizontal line within each box represents the median $CD4^+$ cell percentage, the top and bottom of the box mark the 75^{th} and the 25^{th} percentiles, respectively. The upper and lower bound of the whiskers represent the largest and lowest values within the 75^{th} percentile + 1.5x IQR and the 25^{th} percentile – 1.5x IQR, respectively. Data points beyond these intervals are shown as filled circles and represent outliers. A.) All participants with virologic success are represented in the plot, showing a stable $CD4^+$ percentage recovery after ART initiation. B.) Participants with virologic failure are illustrated within this plot, showing an initial $CD4^+$ percentage recovery, which is less stable than the one from patients without VF, though. The time on ART always includes the interval from the previous month up to the one indicated below the axis (e.g. 6= >0 & ≤6).

Predictors of virologic failure

NNRTI-based ART at the time of analysis (OR=7.32 [95% CI 1.51-35.46], p= 0.013), suboptimal adherence (OR=3.90 [95% CI 1.11-13.68], p= 0.034), and female sex (OR=2.57 [95% CI 1.03-6.45], p= 0.044) were independently associated with VF. Higher CD4 counts (OR=0.20 per additional 10% [95% CI 0.10 - 0.41], p< 0.001) and older age at ART initiation (OR=0.84 per additional year of age at treatment initiation, [95% CI 0.73-0.97], p = 0.017) were protective of VF (Table2).

Table 1. Predictors of virologic failure and acquired HIV drug resistance mutations among children and adolescents attending the Chronic Disease Clinic in Ifakara using logistic regression analysis.

	Virologic Failure						Acquisition of HIV-DRM					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Female	1.45	0.78 – 2.69	0.244	2.57	1.03–6.45	0.044	1.82	0.89 – 3.70	0.101	3.99	1.40 – 11.41	0.010
Current CD4$^+$ percentage per additional 10 percent	0.40	0.26 – 0.62	<0.001	0.20	0.10 - 0.40	<0.001	0.48	0.31 – 0.75	0.001	0.18	0.09 – 0.40	<0.001
CD4$^+$ cell percentage at ART initiation per additional 10 percent	0.97	0.71 – 1.33	0.870	-	-	-	1.15	0.83 – 1.58	0.404	-	-	-
WHO clinical stage 3-4 at ART initiation	0.69	0.36 – 1.33	0.264	0.73	0.29 - 1.86	0.513	0.75	0.36 – 1.55	0.430	0.78	0.27 – 2.21	0.640
Age at ART initiation per each additional year	1.02	0.94 – 1.10	0.671	0.84	0.73–0.97	0.017	0.96	0.87 – 1.05	0.354	0.81	0.68 – 0.95	0.009
Poor adherence[1]	2.39	1.06 – 5.39	0.037	3.90	1.11 - 13.68	0.034	2.15	0.85 – 5.40	0.104	3.72	0.90 – 15.34	0.069
BMI-for-age Z score[2]	1.11	0.86 – 1.42	0.433	0.97	0.70 - 1.35	0.875	1.11	0.84 – 1.47	0.465	0.99	0.69 – 1.41	0.954
Initial ART regimen compared to d4T+3TC+NVP												
AZT+3TC+NVP	1.75	0.74 – 4.11	0.203	2.13	0.56 – 8.10	0.269	1.82	0.65 – 5.08	0.254	2.96	0.69 – 12.79	0.145
AZT+3TC+EFV	0.81	0.37 – 1.77	0.591	1.09	0.36 – 3.30	0.884	1.24	0.52 – 3.00	0.627	1.79	0.50 – 6.41	0.371
Others	1.07	0.26 – 4.40	0.921	1.72	0.23 – 12.58	0.596	1.82	0.42 – 7.80	0.421	3.76	0.45 – 31.30	0.220
NNRTI-based ART regimen compared to PI-based	0.78	0.35 – 1.76	0.553	7.32	1.51 – 35.46	0.013	1.54	0.50 – 4.72	0.450	10.73	1.75 – 65.70	0.010
Orphan (single or double)	0.67	0.32 – 1.41	0.287	0.63	0.21 – 1.86	0.398	0.65	0.29 – 1.49	0.310	0.89	0.26 – 3.04	0.846
ART switch by stock-out	1.20	0.59 – 2.44	0.623	1.39	0.45 – 4.33	0.571	0.99	0.43 – 2.26	0.975	1.38	0.38 – 4.98	0.624
Number of ART switches[3]	1.04	0.94 – 1.16	0.400	0.99	0.82 – 1.20	0.944	1.01	0.90 – 1.14	0.867	0.93	0.75 – 1.16	0.535
Distance to clinic[4]	1.01	0.91 – 1.12	0.854	-	-	-	1.00	0.99 – 1.01	0.825	-	-	-
Transferred to CDCI after treatment initiation	0.77	0.27 – 2.19	0.626	-	-	-	0.83	0.26 – 2.59	0.743	-	-	-

[1] Defined as any missed dose during the last four weeks, reported by the patient or their caregiver

[2] Per each additional unit increase of the z-score

[3] Per each additional number of regimen switch

[4] Per each additional kilometre distance to the hospital

ART: antiretroviral treatment; CTC: care and treatment centre; d4T: stavudine; 3TC: lamivudine; NVP: nevirapine; AZT: zidovudine; EFV: efavirenz, PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; BMI: body mass index; CDCI: Chronic Disease Clinic of Ifakara.

HIV drug resistance mutations

The viral genome was successfully sequenced from 52/54 children and adolescents with VF. Forty-seven (90.4% [95% CI 82.4 - 98.4]) harboured at least one major HIV-DRM at the time of VF. Among patients with HIV-DRM, resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) and NNRTI was found in 80.8% (95% CI 70.1 - 91.5) and 90.2%(95% CI 82.0- 98.4),respectively. Seventy-nine per cent (95% CI 67.7 - 89.9) had major drug resistance against both drug classes. Thirteen patients presented with minor PI-associated drug resistance mutations, which did not limit the activity of any PI, and no major PI-associated drug resistance mutations were found.

The most common resistance mutations were M184V/MV, triggering high-level resistance to 3TC and emtricitabine, found in 40/52 (77%) patients; K103N/KN, causing high-level resistance to efavirenz and NVP, in 25/52 (49%); and Y181C/YC/V, leading to high-level resistance against NVP, in 16/52 (31%). Eighteen patients (34.6%) had detectable thymidine analogue resistance mutations (TAMs), including M41L, D67N, K70R, T215F/Y and K219Q/E, with T215F/Y being the most common and found in 13 (25%) children. Notably, the K65R mutation was present in three adolescents, who had no history of tenofovir disoproxil fumarate (TDF) exposure (Table 3).From the 52 available viral genomes from HIV-1 infected children and adolescents, 44.2% (23/52) were subtype C, 32.7% (17/52) subtype A, 21.2% (11/52) subtype D, and the pol gene of one child (1.9%) consisted of a subtype A protease and subtype D reverse transcriptase. This subtype distribution was consistent with the reported viral subtype circulation in treatment-naïve patients in Ifakara from 2009 (Masimba *et al.*, 2013).

Table 2. Detected drug resistance mutations and respective number of children and adolescents after experienced virologic failure, by drug class.

NRTI-associated DRM	
HIV drug resistance mutations	**Number of patients with mutations**
M184V*	40 (77%)
T215F/Y	13 (25%)
K219Q/E	11 (21%)
K70R	10 (19%)
D67N	9 (17%)
M41L	5 (10%)
T69N/D/G	4 (8%)
K65R, L210W	3 (6%)
A62V	2 (4%)
V75M	1 (2%)
NNRTI-associated DRM	
HIV drug resistance mutations	**Number of patients with mutations**
K103N*	25 (49%)
Y181C*/V	16 (31%)
G190A*	12 (24%)
E138A/G/Q	10 (20%)
K101E/P	9 (18%)
V108I	7 (14%)
A98G	5 (10%)
V90I*, L100I/V, Y188L, P225H*	4 (8%)
K238T	3 (6%)
V106M, V179D, H221Y, M230L	2 (4%)
F227L	1 (2%)

From the 54 patients with VF, 52 were successfully genotyped. Mutations occurring also as mixtures with the wild type sequence are indicated by an asterisk.

Acquisition of drug resistance mutations after treatment initiation

Additional data was available from 44/47 participants with detected HIV-DRM to verify the acquisition of the mutations after ART initiation. Twenty-one (47%) had an available pre-ART plasma sample, which was used to genotype the virus prior to ART exposure. Five of 21 patients harboured DRM but only one child was on an inactive treatment regimen at ART initiation. For another ten patients, we analysed stored samples from their mothers, who were still on a first-line ART. All were virologically suppressed, arguing against a vertical transmission of the respective drug resistant-viruses and suggesting that HIV-DRM emerged only after treatment initiation. Of the remaining 13 children, only $CD4^+$ T cell count and percentage data was available for assessing treatment efficacy. No pre-ART sample of these children had been stored, and their mothers either had been switched to second-line ART or were dead. Eight out of these 13 patients demonstrated stable or increasing $CD4^+$ T cell counts and percentages after treatment initiation, suggesting initial drug activity, thereby further decreasing the likelihood of vertical transmission of a resistant virus. The remaining five children experienced no elevation of $CD4^+$ T cell counts or percentages or even showed declining values, which suggest either poor adherence to ART or transmission of a resistant virus from their mothers. Taken together, these data suggest that at least 86.4% of participants (38/44, 95% CI 76.2 - 96.5) acquired treatment-specific DRM after initiation of ART.

Risk factors for the acquisition of HIV-DRM

Table 2 summarizes the predictors of acquired DRM. NNRTI-based compared to PI-based current regimens (OR=10.73 [95% CI 1.75 – 65.70], p= 0.01) and female gender (OR=3.99 [95% CI 1.40 – 11.41], p=0.01) increased the odds of HIV-DRM. Additionally, poor adherence showed a trend towards increased odds in acquiring HIV-DRM (OR=3.72 [95% CI 0.90 – 15.34], p=0.069). Older age (OR=0.81per each additional year [95% CI 0.68 – 0.95], p=0.009) and higher CD4 percentages (OR=0.18 per additional 10% [95% CI 0.09 – 0.40], p<0.001) showed a protective effect.

Sensitivity and specificity of WHO immunological criteria to detect treatment failure

The current WHO immunological criteria of treatment failure for children ($CD4^+$ T cell count below 200 cells/µL or $CD4^+$ T cell percentage below 10% for children under five years of age and $CD4^+$ T cell count below 100 cells/µL for children aged 5 to 15 years) correctly classified 2/54 (3.7%) children with VF. The sensitivity of the immunological criteria after including adolescents ($CD4^+$ T cell count dropping to the pre-ART level or below) to detect VF rose to 14%. As only one patient would have been mistakenly classified with VF, the specificity of these WHO criteria reached 99.3%.

Discussion

To our knowledge, this is one of the first studies to comprehensively assess VF and the acquisition of HIV-DRM among a large paediatric population in SSA. Key findings are a high rate of both VF and acquired HIV-DRM after a median of over 4 years on ART and an increased risk of both VF and HIV-DRM among participants receiving NNRTI, those with younger age at ART initiation and female patients.

The VF rate of 25.4% exceeded by far the 9.1% failure rate observed for the adult population in the same cohort (Erb, S et al., 2015), emphasizing the great challenge to successfully suppress HIV in paediatric patients. However, the high VF rate found in our study is comparable to previous reports from similar East and West African settings (Kamya *et al.*, 2007; Emmett *et al.*, 2010; Sigaloff *et al.*, 2011; Kebe *et al.*, 2013; Wamalwa *et al.*, 2013; Mutwa *et al.*, 2014; Salou *et al.*, 2016). Of note, it is significantly lower than the actual national Tanzanian average of 38.8% elaborated by the Tanzanian CDC (Ward, J et al., 2014), which could be partially attributed to the specialised counsellors and clinicians in our paediatric unit.

The prevalence of HIV-DRM of 90.4% in children and adolescents with VF in our cohort is comparable with similar settings (Sigaloff *et al.*, 2011; Kebe *et al.*, 2013; Salou *et al.*, 2016) and the adult population in our clinic [32]. The multi-class resistances, present in almost 80% of all failing patients dramatically limits future treatment options and represents an important public health concern. The presence of the K65R mutation in three patients is also concerning, as this triggers resistance to TDF and abacavir (but not zidovudine) again limiting future treatment options. The observed prevalence

of TDF-resistances in our setting was, however, much lower than in a recently published multicentre cohort study of patients failing to first-line ART (TenoRes Study Group, 2016). Interestingly, the three patients with the multi-class resistance triggering K65R mutation had not been exposed to TDF. As already highlighted in other studies, stavudine (compared to zidovudine) may also select this multi-class resistance mutation (Nouhin *et al.*, 2013). Although stavudine is currently hardly used, this should be taken into account in patients previously treated with this drug. The absence of major PI mutations is reassuring. As PIs have a short half-life and a higher barrier to resistance, it has been suggested that they are less likely to allow the emergence of drug resistance mutations in early VF (Walmsley *et al.*, 2002; Bangsberg, Moss and Deeks, 2004).Of note, patients on PI-based second-line treatment also received additional counselling. Overall, these results indicate that PI-based second-line regimens potently suppress HIV in children and adolescents. However, new drug classes such as integrase inhibitors and new paediatric drug combinations are strongly needed in this setting to be able to treat individuals with multi-class resistant virus. Even children with active second-line regimen will eventually depend on new drug classes as they rely on ART for decades, with growing risk to fail on the second-line. Although recycled NRTIs show residual activity in adult populations (SECOND-LINE Study Group *et al.*, 2013; Paton *et al.*, 2014), these results cannot be extrapolated to paediatric populations.

In 86.4% of the patients with HIV-DRM, the resistant viral variants likely emerged after ART initiation. However, as imperfect adherence of the mother during pregnancy would lead to the possible archiving of drug resistant proviruses, it cannot be excluded that in these cases viral minorities carrying resistance-associated mutations were also transmitted to the offspring. Such virus would then only emerge once drug pressure with the respective drug was applied. As stated above, although this possibility cannot completely be ruled out, our analysis renders this explanation unlikely for most of the observed resistance cases: no recorded failure of the mother, initial period of treatment success in the child, and paediatric regimen different from the mother's therapy.

Poor adherence predicted VF and showed a trend to predicting the acquisition of HIV-DRM. Adherence is dependent on drug, social, health system and health workers' factors (Bikaako-Kajura *et al.*, 2006; Sutcliffe *et al.*, 2008). The development of new child-friendly drug formulations is needed. In addition, a functioning procurement and distribution system is crucial and tools to facilitate the prescription of paediatric drugs need to be widely disseminated and routinely used. In our cohort, the number of treatment switches significantly increased the odds for poor adherence (by 58%) and for being under-dosed for each additional change of treatment (by 50%) (Table S1). It is essential to minimise treatment switches in this context to improve adherence.

Table S1. Predictors for poor adherence (n=119) and being under-dosed (n=54) among children and adolescents from the Chronic Disease Clinic in Ifakara (CDCI).

	Poor adherence						Under-dosage					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Female sex	0.66	0.29 – 1.48	0.311	0.75	0.18 – 3.04	0.684	0.44	0.14 – 1.38	0.158	0.78	0.17 – 3.56	0.743
CD4$^+$percentage per additional 10 percent	0.75	0.49 – 1.16	0.200	1.32	0.60 – 2.90	0.494	-	-	-	-	-	-
WHO clinical stage 3-4 at ART start	1.79	0.76 – 4.21	0.186	1.49	0.40 – 5.57	0.554	0.45	0.13 – 1.52	0.199	0.24	0.05 – 1.25	0.091
Age at ART start per additional year	1.12	1.01 – 1.24	0.039	1.34	1.04 – 1.71	0.021	0.87	0.76 – 1.01	0.059	0.73	0.57 – 0.92	0.007
BMI-for-age Z score[1]	1.12	0.81 – 1.56	0.497	0.98	0.58 – 1.65	0.943	0.82	0.54 – 1.25	0.343	0.90	0.50 – 1.61	0.711
Initial ART regimen compared to d4T+3TC+NVP												
AZT+3TC+NVP	0.43	0.11 – 1.61	0.208	0.13	0.01 – 2.42	0.169	-	-	-	-	-	-
AZT+3TC+EFV	0.90	0.38 – 2.17	0.821	2.90	0.66 – 12.86	0.161	-	-	-	-	-	-
Current ART regimen compared to PI-based												
TDF-based 1st-line	1.56	0.38 – 6.47	0.538	5.48	0.47 – 64.64	0.177	-	-	-	-	-	-
EFV-based 1st-line	1.30	0.37 – 4.58	0.685	2.39	0.29 – 19.99	0.421	-	-	-	-	-	-
NVP-based 1st-line	1.06	0.31 – 3.58	0.928	3.43	0.40 – 29.52	0.262	-	-	-	-	-	-
Vital status of parents compared to both alive												
Double orphan	1.38	0.40 – 4.71	0.608	4.70	0.65 – 34.13	0.126	-	-	-	-	-	-
Maternal orphan	0.99	0.31 – 3.20	0.990	0.18	0.03 – 1.29	0.088	-	-	-	-	-	-
Paternal orphan	0.86	0.24 – 3.03	0.808	0.48	0.07 – 3.40	0.465	-	-	-	-	-	-
ART stop or change due to drug stock-out	0.64	0.23 – 1.79	0.397	0.60	0.11 – 3.20	0.547	-	-	-	-	-	-
Number of ART switches[2]	1.14	1.01 – 1.28	0.028	1.58	1.20 – 2.07	0.001	1.21	1.00 – 1.47	0.048	1.53	1.12 – 2.08	0.008
Distance to clinic[3]	1.00	0.99 – 1.01	0.976	0.99	0.97 – 1.01	0.439	-	-	-	-	-	-

[1] Per each additional unit increase of the z-score

[2] Per each additional number of regimen switch

[3] Per each additional kilometre distance to the hospital

ART: antiretroviral treatment; CTC: care and treatment centre; d4T: stavudine; 3TC: lamivudine; NVP: nevirapine; AZT: zidovudine; EFV: efavirenz, PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; BMI: body mass index; CDCI: Chronic Disease Clinic of Ifakara.

Younger age at ART start, NNRTI-based regimens and female sex were identified as risk factors for VF and the acquisition of HIV-DRM. Sub-therapeutic drug levels in younger children due to difficulties to administer the drugs, faster metabolism, differences in pharmacokinetics and dose-prescribing errors could explain this finding (van Rossum, Fraaij and de Groot, 2002; Menson *et al.*, 2006; Ellis *et al.*, 2007; Wamalwa *et al.*, 2013). Additional sub-group analysis revealed that 35.2% (19/54) of the study participants with VF were prescribed a drug dose below the recommended for the patient's body weight at least once since ART was initiated, as previously highlighted by a study among this same paediatric population (Gamell, Muri, *et al.*, 2016). Of note, after a dedicated paediatric unit had been established in our clinic, such dosing errors were eliminated. Children and adolescents on NNRTI-based regimens had much higher odds to experience VF and to acquire DRM than participants on PI-based regimens, again suggesting the good performance of PI regimens in suppressing HIV and preventing DRM. As mentioned above, the additional counselling in patients on PI-based second-line treatment might have slightly biased this finding. Girls were more vulnerable to VF and the development of HIV-DRM for reasons that could not be explained in the framework of this study, but indeed sex inequalities were also found in other recent studies from East Africa (Lazzerini *et al.*, 2016).

The WHO criteria for immunological failure showed an alarmingly low sensitivity of 5% in children and 14% in adolescents. For most of the study participants with VF, the $CD4^+$ T cell counts stayed above 20% and 500 cells/µL after initial immune cell recovery, values considerably higher than the WHO recommended criteria for immunological failure (WHO, 2013). A similar study from Rwanda revealed that even a threshold of <350 $CD4^+$ T cells/µL to detect treatment failure had a very low sensitivity ranging from 19% to 32% (Mutwa *et al.*, 2014). The late detection of patients with treatment failure leads to the accumulation of drug resistances and dramatically limits treatment options (Sigaloff *et al.*, 2011, 2012). The implementation and up-scaling of VL monitoring is essential to maintain treatment options and optimize health outcomes in resource-limited setting with restricted treatment possibilities (Sutcliffe *et al.*, 2008; Sigaloff *et al.*, 2011; Salou *et al.*, 2016).

A limitation of this study was the definition of VF as a single VL ≥1000 copies/mL, which has a lower sensitivity than the official WHO definition for VF. It is possible that children with lower VL also carry DRM, though. Furthermore, the use of population sequencing for genotyping might have led to an underestimation of drug resistance mutations also in pre-ART samples. Our

assessment for acquisition of DRM compared to transmitted DRM may have allowed some transmitted DRM to remain unnoticed by sequencing pre-ART samples. In addition, a suppressed VL of the mother on first-line treatment does not ultimately exclude transmission of DRM.

In conclusion, our study found high rates of VF and emerging HIV-DRM in this paediatric population on long-term ART in rural Tanzania. Both VF and the emergence of HIV-DRM were associated with NNRTI use, younger age at ART initiation, poor adherence, and female sex. Moreover, our results reinforce the current knowledge about the low sensibility of the WHO criteria for immunological treatment failure in children and adolescents. These findings provide relevant information for clinicians and health policy makers and raise concerns about the effectiveness of current paediatric ART programmes in SSA, calling for a critical review of current guidelines. In particular, awareness needs to be raised in order to advocate for the strengthening of adherence strategies tailored to this vulnerable population, the development and widespread availability of new paediatric ART formulations, and the universal roll-out of routine VL monitoring for the millions of children and adolescents living with HIV in SSA.

Acknowledgements

The authors are grateful to all children andfamilies attended in the One Stop Clinic and the staff from the Chronic Diseases Clinic of Ifakara. Also, we want to thank the funders of our clinic: the Ministry of Health and Social Welfare of Tanzania, the Swiss Tropical and Public Health Institute, the Ifakara Health Institute, the Government of the Canton of Basel, USAID through the local implementer TUNAJALI-Deloitte, and the Merck for Mothers Global Giving Program.

Our special thanks is extended to all members of the KIULARCO Study Group: Aschola Asantiel, Manuel Battegay, AdolphinaChale, Diana Faini, Ingrid Felger, Gideon Francis, Hansjakob Furrer, Anna Gamell, Tracy Glass, Christoph Hatz, Speciosa Hwaya, Aneth Vedastus Kalinjuma, Bryson Kasuga, Namvua Kimera, Yassin Kisunga, Thomas Klimkait, Emilio Letang, Antonia Luhombero, Lameck B Luwanda, Herry Mapesi, Leticia Mbwile, Mengi Mkulila, Julius Mkumbo, Margareth Mkusa, Dorcas Mnzava, Germana Mossad, Dolores Mpundunga, Daimon Msami, Athumani Mtandanguo, Kim D Mwamelo, Selerine Myeya, Sanula Nahota, Regina Ndaki, Agatha Ngulukila, Alex John Ntamatungiro, Leila Samson, George Sikalengo, Marcel Tanner, Fiona Vanobberghen and MajaWeisser.

Funding

This work was supported by the funders of the Chronic Diseases Clinic of Ifakara and its paediatric and PMTCT unit, the One Stop Clinic: the Ministry of Health and Social Welfare of Tanzania; the Swiss Tropical and Public Health Institute; the Ifakara Health Institute; the Government of the Canton of Basel; USAID through its local implementer TUNAJALI-Deloitte; and the Merck for Mothers Global Giving Program.

Conflict of interests

The authors declare no conflict of interest for their role in the study and for the writing of the manuscript.

10. Discussion

The five studies presented in this thesis contribute to the understanding of the challenges of delivering PMTCT and paediatric HIV services in a rural sub-Saharan African setting, demonstrate the possibility of delivery changes, and measure the impact of a comprehensive service delivery model. The studies were designed to overcome service delivery barriers and improve the clinical care of HIV-infected mothers, children, and their families. Moreover, the acquisition of drug resistance-associated mutations among HIV-infected children, which is an emerging public health concern, is comprehensively characterized.

The findings of this work are particularly timely, given the global commitment to eliminate new HIV infections among children, ensure that their mothers remain healthy and improve the HIV diagnosis and treatment for children. In African rural areas barriers to access HIV care are more evident and it is in these settings where innovative models to deliver HIV care are more needed. In addition, lessons learned from the implementation of Option B+ are of most value considering the recent evolution towards “test and start” for all PLWHIV.

10.1. Strengths and limitations of the work included in this thesis

This thesis is the result of an initial evaluation of the PMTCT implementation, one of the most critical interventions to control the HIV/AIDS pandemic in sub-Saharan Africa, followed by the design and evaluation of the impact of a strategy to improve the service delivered to HIV-infected pregnant women, mothers, children and their families. In addition, during the execution of this operational research work, a potential threat to paediatric ART programs in Africa could be identified and comprehensively characterised.

One of the absolute strengths of this work is that through an operational agenda, real changes in the HIV care delivery model were implemented and sustained in Ifakara. The goal of a MTCT below 5% was achieved. The Chronic Diseases Clinic of Ifakara is an invaluable platform to evaluate the impact of new policies on clinical outcomes with its open, ongoing cohort. The prospective character of the key studies and the comprehensive clinical data presented represent a main strength of the findings presented in this thesis. Study 3 is the first one to comprehensively analyse the Option B+ cascade in a Tanzanian setting. The analysis included all the steps of the PMTCT pathway and a long follow-up for both mothers and infants. Likewise, Study 5 is one of the

first studies to carefully assess virological failure and the acquisition of HIV drug resistance mutations among a large paediatric population in sub-Saharan Africa.

The studies included in this work do have some limitations. First, a longer follow-up will be necessary to measure the long-term impact of the strategy on the clinical outcomes and retention in care. We consider this as a call for future research. Second, as interventions to improve maternal and paediatric HV care were implemented as a bundle, we cannot distinguish the impact of each specific intervention. Third, since 14% of HIV-exposed infants were lost to follow-up while breastfeeding, the reported 2.2% MTCT rate possibly underestimated the true HIV transmission rate. However, even presuming a 15% transmission through the breast milk among infants lost, the cohort MTCT rate would still be below 5%. Finally, for Study 5 we used as definition of virological failure a single viral load above 1000 copies/mL, and it is possible that children with lower viremia also carried drug resistance mutations. Future studies should conduct longer follow-up in this ongoing cohort to measure the long-term impact of the strategy on the clinical outcomes and retention in care

10.2. Discussion of key findings

The initial PMTCT assessment, gaps identified and the strategy developed to improve maternal and paediatric HIV care in Ifakara

Several gaps were identified along the PMTCT pathway implemented in Ifakara during the period 2010 – 2011. All services and most resources needed for a proper functioning of the PMTCT program recommended by the Tanzanian authorities were in place, but major gaps prevented an optimal implementation. These gaps included: a) no re-testing of seronegative pregnant women during late pregnancy; b) poor linkage of HIV-infected pregnant women to HIV care; and c) lack of a standardized follow-up for HIV-exposed infants.

Over 90% of women were tested for HIV during the antenatal care visits, but seronegative women were very rarely re-screened in late pregnancy. Similar findings are reported from South Africa (Technau *et al.*, 2014). In HIV high-prevalence settings, re-testing for HIV in the third trimester is recommended based in its cost-effectiveness (Soorapanth *et al.*, 2006) and the much higher MTCT risk among women with incident HIV infection (Drake *et al.*, 2014). Studies from Botswana and South Africa have reported a rate of seroconversion during pregnancy of 1.3% and 4.2% respectively (Lu, L et al., 2011; Technau *et al.*, 2014). In the absence of a maternal diagnosis,

infants cannot be captured by EID programs. HIV diagnosis and treatment initiation for these infants will consequently be delayed, with a significant effect on morbidity and mortality.

During the first assessment of the PMTCT care pathway in Ifakara, only 25.6% of pregnant women newly-diagnosed with HIV in the antenatal care were enrolled to HIV care. This finding was especially surprising since the HIV clinic in St Francis Referral Hospital is located only few meters away from the antenatal care clinic. Reasons for not being linked into HIV care may have been related to poor post-test counselling and the perceived stigma of being seen while pregnant at the HIV clinic. Studies from neighbouring countries have reported non-disclosure, stigma, lack of male partner support and health worker attitudes as barriers to linkage to HIV care after testing positive in the antenatal care (Mugasha *et al.*, 2014; Saleem, Kyeyagalire and Lunsford, 2014). The PMTCT guidelines at the time recommended different antiretroviral regimens depending on the immunological and clinical presentation (Tanzanian MoHSW, 2007b). Not being enrolled in HIV care meant that most women were not assessed for ART eligibility and even if a single antiretroviral drug was given during pregnancy, no postpartum follow-up existed.

Another major gap identified was the absence of a standardised follow-up for HIV-exposed infants. Infants could not be ensured a correct postnatal antiretroviral prophylaxis, feeding counselling, and the performance of an EID test. It was thus impossible to estimate the MTCT rate.

At the time of the first study, the uptake of guidelines in sub-Saharan Africa was known to be poor. This was partly due to the fragility of health systems, lack of infrastructure, and constrains on human and financial resources (Doherty, McCoy and Donohue, 2005; Nkonki *et al.*, 2007; Both and van Roosmalen, 2010). Dissemination of changes in the guidelines was suboptimal and it often took time to be rooted among health workers and to change practices. According to WHO, in 2009, only 53% of pregnant women worldwide received any antiretroviral drug for PMTCT (WHO, 2010c), with substantial differences across countries in sub-Saharan Africa (i.e. 1% in the Democratic Republic of the Congo versus 52% in Mozambique). It was thus clear that having effective antiretroviral regimens was insufficient and effective delivery programs were equally important (Barker, Mphatswe and Rollins, 2011; Ciaranello *et al.*, 2012).

This first study illustrates that it is possible and important to measure the quality of the PMTCT provision in a rural setting like Ifakara, by providing a cross-sectional snapshot of the reality in the field. Gaps can be identified and potential ways to bridge them at an operational level can be

analysed. The study highlighted the need for much simpler and effective PMTCT programs, as Option B+. Moreover, the findings of this first assessment set the basis for the development of a new strategy to be tested in a prospective fashion with the aim of improving the care of HIV-infected mothers and their families in rural Tanzania and other similar settings in sub-Saharan Africa.

From December 2012 onwards, a bundle of measures were implemented at the Chronic Diseases Clinic of Ifakara to improve the HIV services delivered to PLWHIV. Measures affecting all patients were the implementation of electronic medical records in the HIV clinic and provider-initiated HIV testing and counselling in the hospital wards. Measures specifically targeting HIV-infected pregnant women, children and adolescents and their families, were the creation of a maternal and paediatric HIV unit integrated within the reproductive and child health clinic and the performance of pro-viral HIV DNA PCR locally. The maternal and paediatric unit was named the "One Stop Clinic of Ifakara". Also, in September 2013, the Ministry of Health and Social Welfare changed the PMTCT guidelines and recommended Option B+. The new recommendations were progressively rolled out through the different districts, and in Ifakara were implemented through the One Stop Clinic from April 2014.

Impact of the One Stop Clinic and the accompanying measures on the care provided to HIV-infected pregnant women, children and their families

To assess the impact of the One Stop Clinic in combination with the other measures and the national implementation of Option B+, two studies (Study 2 and Study 3) were designed. Study 2 compares the diagnosis, linkage to care, treatment coverage and retention of HIV-infected pregnant women and children before (2008 - 2012) and after (2013 - 2014) the One Stop Clinic. Study 3 describes the uptake of PMTCT Option B+ recommendations during its first year of implementation (4/2014 - 3/2015) and compares it with the previously described in Study 1, before the simplification of the guidelines and the integration of maternal and paediatric HIV care within the reproductive and child health clinic.

The combined results of these two studies showed that the bundle of measures put into practice to improve maternal and paediatric HIV care in Ifakara in 2013, along with the national roll-out of Option B+ later in 2014 resulted in: a) increased number of pregnant women and children diagnosed and linked into care; b) increased detection of children with AIDS; c) ART coverage close to 100% among eligible children and pregnant women; d) improved retention in care; and e) MTCT

below the national average (9%) and below the 5% threshold established for elimination of MTCT of HIV in breastfeeding populations. Nevertheless, gaps such as the poor uptake of HIV testing in the labour ward and the almost inexistent HIV re-screening during late pregnancy remained. Also, the need for approaches to timely diagnose HIV-infected children outside the PMTCT program was made evident.

Improvements of the PMTCT cascade and persisting challenges

The strategy implemented in Ifakara resulted in an increased number of pregnant women diagnosed and linked into care. The HIV testing rate in the antenatal clinic was over 90% and linkage to care of newly diagnosed HIV-infected pregnant women, increased from 25.6% to 92%. Studies from neighbouring countries analysing the improvements after Option B+ implementation present inconsistent results. In two large urban centres in Malawi, the enrolment to PMTCT/HIV care from the antenatal care clinic increased from 61% in the pre-B+ period to 87% in the post-B+ period (Kim *et al.*, 2015). However, in Uganda, only 25% of women diagnosed in rural antenatal care settings during the post-B+ period were linked to HIV care (Mugasha *et al.*, 2014). Thus, we believe that the improvement achieved in Ifakara cannot be solely attributed to Option B+ itself, but to the integration of services and the counselling delivered through the One Stop Clinic.

As much as the above achievements have to be celebrated, the unreliable HIV re-screening in late pregnancy and the low HIV testing uptake in the labour ward in Ifakara are worrisome. As already exposed above, in high prevalence settings, HIV seroconversion during pregnancy is not uncommon. Vertical transmission of HIV during primary infection is significantly increased. Thus, educational efforts are needed to implement HIV re-screening as routine practice in antenatal care clinics and maximise the benefits of PMTCT. Maternity and labour wards are also critical entry points to PMTCT that need attention. HIV testing uptake at delivery rooms has been reported to be low in African settings (Beltman *et al.*, 2010; Ononge *et al.*, 2014) and represent a missed opportunity to capture mother-infants pairs into PMTCT/EID. To mitigate these persisting gaps continuous training of health providers coupled with a functioning supply of HIV tests is needed.

ART coverage among pregnant women attended at the One Stop Clinic under Option B+ recommendations was 90.6%. Importantly, two different subgroups of women have to be considered: (i) women under HIV-care who became pregnant; and (ii) women newly-diagnosed with HIV during pregnancy. Treatment coverage for the first group was 100%, but for the second group it was 75.5%. In our setting, an important proportion of newly diagnosed pregnant women

were lost along the very initial steps of the PMTCT circuit: 8% were not linked to HIV care after HIV diagnosis, and among those who did enrol, 18% did not return for completing pre-ART counselling and initiate treatment. Similar early attrition rates have been observed in other sub-Saharan Africa settings such as Malawi, where Option B+ was first introduced. A study comparing different models of Option B+ delivery found that in places where antenatal care and provision ART are located within the same health facility, approximately 20% of HIV-positive women visited at the antenatal care did not start ART (van Lettow *et al.*, 2014). Also in Malawi, in two large urban health centres where HIV-infected women are initiated and followed on ART at the antenatal care clinic, the attrition rate before treatment initiation was 18% (Kim *et al.*, 2015). Remarkably, data from rural health centres in the country showed a significantly lower ART uptake, with 44% of HIV-infected women enrolled in antenatal care not starting treatment (Chan *et al.*, 2016). These findings clearly indicate that largely asymptomatic pregnant women may need time to adjust to the HIV diagnosis and understand the benefits of lifelong treatment.

After ART initiation, recently diagnosed women continued to drop from care, although at a lower rate: 86.5% were retained after a median of 17.2 months. This result is better than the one reported in a recent publication from Malawi, where retention after ART initiation in the context of Option B+ was 68.5% at 12 months, 61% at 24 months and 56.3% at 36 months (Haas *et al.*, 2016). Studies exploring the factors associated with lost to follow-up among women started on ART for PMTCT found that common reasons for stopping ART included travel, lack of transport money, poor pre-ART counseling and drugs' side effects (Tweya *et al.*, 2014; Ebuy, Yebyo and Alemayehu, 2015). The better results from Ifakara suggest that the unhurried start of ART and continued counselling combined with joint visits for postpartum care, PMTCT/HIV care and infant growth monitoring and immunization are responsible for the lower attrition. Retention among women becoming pregnant while being under HIV care was clearly better, with 90% being retained after a median follow-up of 19 months.

It is reported in the literature that whilst integration of services increases the rates of linkage to HIV care and ART start, retention after treatment initiation is significantly lower in comparison to settings where ART is provided outside the antenatal care (van Lettow *et al.*, 2014; Chan *et al.*, 2016). This represents a downside of the full integration of PMTCT and antenatal care services. Furthermore, pregnant women who initiate ART on the same day of HIV diagnosis are less likely to return for a follow-up visit (Tenthani *et al.*, 2014). These findings most probably show that, in a

single antenatal visit, women are not able to fully comprehend the consequences of a positive HIV test and the initiation of a lifelong treatment (Medley *et al.*, 2004; de Bruyn and Paxton, 2005; Gebrekristos, Mlisana and Karim, 2005). To optimize the uptake of PMTCT throughout the pregnancy and breastfeeding periods, further operational research must focus on the optimal timing between HIV diagnosis and ART initiation and the counselling that is coupled with it. In addition, the better retention of women becoming pregnant while being under HIV care must be capitalized and peer-mother programs should be common in all sub-Saharan African settings (Tenthani *et al.*, 2012; Shroufi *et al.*, 2013; Foster *et al.*, 2017).

The elimination of mother-to-child transmission can be achieved in rural Africa

The current MTCT rate in Ifakara is 2.2%. The <5% target established for populations in which breastfeeding is common (WHO, 2014a) has been achieved. Therefore, unlike previously reported (Wudineh and Damtew, 2016), we have shown that the virtual elimination of new paediatric HIV infections is feasible in a rural setting under programmatic circumstances.

After the initial assessment of the PMTCT cascade, a standardized follow-up for HIV-exposed infants was organized and allowed for the description and outcomes analysis of this population. HIV-exposed infants are enrolled at the One Stop Clinic through two main different entry points. On one side there are infants identified through the PMTCT program. These are infants born from mothers enrolled in HIV care before delivery, either because they became pregnant while being under HIV care, either because they were diagnosed with HIV during the antenatal visits. On the other side, some infants younger than 18 months are identified through provider-initiated HIV testing and counselling, mostly when admitted in the hospital wards with symptoms. In most of these cases, mothers were not tested for HIV during pregnancy and are also diagnosed then.

HIV-exposed infants enrolled from the PMTCT program in Ifakara had a virological test done at a median age of six weeks. Most of them (91%) were exclusively breastfeed, and had very low attrition, with a lost to follow-up rate of only 14% after a minimum of 15 months since enrolment. This is one of the highest retention rates described in sub-Saharan Africa, where the reported average lost to follow-up rate at only 3 months post-delivery is 34% (Sibanda *et al.*, 2013). Studies reporting on retention of HIV-exposed infants at 12 - 18 months of age have reported losses of 27% in Kenya (Nyandiko *et al.*, 2010), 40% in urban South Africa (Chetty *et al.*, 2012), and 24% in Zimbawe (Kurewa *et al.*, 2011). The high retention in Ifakara must be attributed to the integrated service delivery model, with mother–infant pairs receiving different health services in one single

day and at one single place, the shortened turn-around time of EID results, and the continuous counselling that women receive during pregnancy and breastfeeding periods. To further improve retention and HIV-free survival of exposed infants, additional interventions with proven efficacy as mobile phone-based reminders and the promotion of male partner involvement should also be implemented in our setting (Ambia and Mandala, 2016).

These successful results are shadowed by the number of children younger than 18 months that were identified to be HIV-exposed during hospital admission. Through provider-initiated HIV testing and counselling these infants and their mothers were enrolled in HIV care, but for many, prevention measures to avoid vertical transmission of HIV could not be offered: 46% of the infants were already HIV-infected. This highlights once more the value of testing for HIV infants and children who seek for health care. This finding is also an evidence that in a region where 95% of women attend antenatal care (Tanzanian NBS, 2011), some women are not captured by PMTCT programs and drives us back to the testing gaps observed in late pregnancy and labour ward.

Increased detection of children living with HIV, universal ART coverage and improved retention in care

The number of children enrolled in care increased after the One Stop Clinic was established. Multiple factors are responsible for this finding. The implementation of provider-initiated HIV testing and counselling in the hospital wards is the main one. The performance of EID test locally, and the family-centred testing using parents as index cases also contributed to the increased identification of children living with HIV. Importantly, children diagnosed after the bundle of measures implementation, presented with more advanced disease and immunosuppression, reflecting the high yield of infants and children enrolled from the in-patients wards. Since the incidence of HIV has decreased over the last years in Tanzania, we presume that before the intervention, some children were admitted and eventually died without being diagnosed with HIV and therefore enrolled in our cohort. Diagnosing and offering health-restoring care and treatment to children with AIDS is one of the major achievements of our strategy. On the other hand, it highlights the enormous challenge that diagnosing children outside PMTCT programs represents. Beyond PMTCT/EID programs, evidence on the most effective strategies to identify and link HIV-infected children to care is scarce (Horwood *et al.*, 2010; Chamla *et al.*, 2013; Busza *et al.*, 2014). Case-finding approaches targeting places where large number of infants and children congregate are much needed (Ahmed *et al.*, 2013; Essajee *et al.*, 2017; Penazzato *et al.*, 2017). Testing in

places where children at high risk of HIV seek for health services is effective. Provider-initiated testing and counseling on inpatient wards has been shown to be an efficient approach to identify HIV-infected children in high HIV prevalence settings (Weigel *et al.*, 2009; McCollum *et al.*, 2011; Preidis *et al.*, 2013; Ferrand *et al.*, 2016) but is rarely implemented routinely (Kranzer *et al.*, 2014). Children identified at paediatric wards, malnutrition units and tuberculosis clinics present with advance disease, and their treatment outcomes could have been better if identified before. Thus, along provider-initiated HIV testing in health care settings, new ways to promptly diagnose children must be sought.

ART coverage among enrolled children according to the guidelines at each time period was assessed before and after the establishment of the One Stop Clinic. During the period 2008 - 2012 79.6% of children meeting criteria were prescribed ART. After 2012, this rate raised to 98.1%. This universal coverage is remarkable since similar coverage rates in Africa had only been reported by urban programs (Anaky *et al.*, 2010; Leyenaar *et al.*, 2010).

After enrolment and eventual ART initiation, it is crucial that children remain in care. Early retention in paediatric sub-Saharan African cohorts is significantly affected by mortality. Advanced HIV disease at presentation, often accompanied with severe malnutrition, systemic bacterial infections and opportunistic infections as tuberculosis or *Pneumocystis jirovecii* pneumonia, is associated with early mortality after enrolment (Eley *et al.*, 2006; Bolton-Moore *et al.*, 2007; Bong *et al.*, 2007; Reddi *et al.*, 2007; Anaky *et al.*, 2010; Leyenaar *et al.*, 2010; McConnell *et al.*, 2010; Koller *et al.*, 2015). In our cohort, in 2013 - 2014, mortality ascertainment six months after enrolment was 15.7%. All deaths occurred among children diagnosed through provided-initiated HIV testing and counselling, after they presented at the hospital seeking for health care and almost all were malnourished. Analogous findings have been reported from Malawi, where 12-months mortality after diagnosis through provider-initiated HIV testing was 20% (McCollum *et al.*, 2011), and Zambia, where mortality among malnourished children was 46%, with HIV-infected children being 80% more likely to die (Munthali *et al.*, 2015). This high mortality is the consequence of a series of barriers and missed opportunities that impeded, in first place, the prevention of vertical infection and, later, the provision of early diagnosis and life-saving treatment.

Uncovering and analysing the problem of virological failure and drug resistance mutations among children

After the establishment of the One Stop Clinic, a reduced team of clinicians, nurse and counsellor started to take care of all children and adolescents of KIULARCO as well as their HIV-infected relatives. We organized a child and family-friendly unit, implement flexible age-based clinic days, and arrange activities to deliver tailored health education messages as group disclosing sessions and teen clubs. Moreover, a patient-health provider relationship could be established with a consequent closer follow-up and better understanding of the patients' family and social circumstances. This child and family-centred approach drew attention to some problems that were previously unnoticed.

The most relevant of these uncovered issues was the impression that children presented treatment failure more often than adults and that failure was recognized late. To explore this perceived problem, we first described a well-characterised series of children and adolescents presenting treatment failure and acquired HIV drug resistance-associated mutations (Study 4). Later on, we designed Study 5, a longitudinal study to assess the prevalence and predictors of virological failure and drug resistance mutations among children.

The case series (Study 4) raised concerns about a scarcely reported emerging public health concern in sub-Saharan Africa. By the time of publication, only one study from Kenya had described the pattern of acquired drug resistance mutations in children failing to ART in Africa (Wamalwa *et al.*, 2013). Factors known to be related to virological failure, such as suboptimal adherence, non-parental caregiver, and ART regimen switches (Sigaloff *et al.*, 2011; Zoufaly *et al.*, 2013; Mutwa *et al.*, 2014) were common among our patients in Ifakara. Furthermore, we identified a frequently overlooked factor: the prescription of inadequate doses of antiretrovirals. This last factor indicates that awareness needs to be raised among health workers and tools to facilitate the prescription of paediatric drugs need to be widely disseminated and routinely used (ICAP, 2006). Another relevant finding was the confirmation that the WHO's clinical and immunological criteria failed to identify most children presenting treatment failure and already multi-class resistance mutations, thus emphasising the urgent need for routine viral load monitoring.

Study 5 is one of the first studies to comprehensively assess virological failure and the acquisition of drug resistance-associated mutations among a large paediatric population in sub-Saharan

Africa. Key findings are a high rate of both virological failure and acquired HIV drug resistance mutations after a median of over four years on ART and an increased risk of both among participants receiving NNRTI-based regimens, those with younger age at treatment initiation and female patients.

The virological failure rate of 25.4% found in our study is comparable to previous reports from Eastern and Western Africa (Kamya *et al.*, 2007; Emmett *et al.*, 2010; Sigaloff *et al.*, 2011; Kebe *et al.*, 2013; Wamalwa *et al.*, 2013; Mutwa *et al.*, 2014; Salou *et al.*, 2016), but exceeded by far the 9.1% failure rate observed among adults in the same cohort (Erb *et al.*, 2017). The only Tanzanian data available for comparison are the unpublished results of a study presented by the Tanzanian CDC at a national conference. They reported an average virological failure rate of 38.8% among children (Ward, J et al., 2014). Our lower virological failure prevalence can be partially attributed to the specialised counselling and clinical team of the One Stop Clinic, but it is still alarmingly high when compared with the observed failure rates among adults.

Similarly, the 90.4% prevalence of drug-resistance mutations among failing children and adolescents is comparable with other African settings (Sigaloff *et al.*, 2011; Kebe *et al.*, 2013; Salou *et al.*, 2016) and the adult population from KIULARCO (Ntamatungiro *et al.*, 2017). Almost 80% of all patients with drug resistance-associated mutations present multi-class resistances, limiting future treatment options and representing an important public health concern. The absence of major PI mutations is reassuring and indicates that PI-based second-line regimens potently suppress HIV in children and adolescents. However, new drug classes such as integrase inhibitors and new paediatric drug combinations are strongly needed in sub-Saharan African settings to treat individuals with multi-class resistant virus. Even children with an active second-line regimen will eventually depend on new drug classes as they rely on ART for decades, and with time they may fail to the second line.

Our findings suggest that at least in 86.4% of patients with drug resistance mutations, the resistant virus variants emerged after ART initiation; mutations were acquired while on treatment, not transmitted from the mothers. We acknowledge that since we used population sequencing for genotyping, our assessment for acquisition of mutations may have allowed some transmitted mutations to remain unnoticed by sequencing pre-ART samples. However, our analysis renders this explanation unlikely for most of the observed cases: no recorded failure of the mother, initial

period of treatment success in the child, and paediatric regimen different from the mother's therapy.

Adherence to treatment and availability of paediatric drugs

Optimal treatment outcomes can be predominantly ensured if children adhere to available, effective, correctly dosed, simple and child-friendly drugs. However, such drugs are not widely available in sub-Saharan Africa and treatment compliance among children and adolescents can be complex. In our study, poor adherence predicted virological failure and showed a trend to predict the acquisition of drug resistance mutations. Further analysis of our data revealed that the number of treatment switches significantly increased the odds for poor adherence and for prescription of low-dose of antiretrovirals. Adherence among children depends on drug, social, family, health system and health workers factors (Sutcliffe *et al.*, 2008; Nachega *et al.*, 2009; Fetzer *et al.*, 2011; Bernays *et al.*, 2014). Interventions to support children and families, a functioning procurement and distribution system, and tools to facilitate the prescription of paediatric drugs are much needed (Luyirika *et al.*, 2013; Lowenthal *et al.*, 2014). Furthermore, more efforts have to be put to develop new child-friendly formulations, with fixed-dosed combinations that allow weight-band dosing, minimize the number of drugs needed and, in turn, improve procurement and availability (Penazzato *et al.*, 2015).

Predictors of virological failure and acquisition of drug resistance mutations

Younger age at ART start, NNRTI-based regimens and female sex were identified as risk factors for virological failure and the acquisition of HIV drug resistance-associated mutations. Sub-therapeutic drug levels in younger children due to difficulties to administer the drugs, faster metabolism, differences in pharmacokinetics and dose-prescribing errors could partly explain these findings (van Rossum, Fraaij and de Groot, 2002; Menson *et al.*, 2006; Ellis *et al.*, 2007; Fillekes *et al.*, 2011; Wamalwa *et al.*, 2013). Prescribing correct doses of drugs to young infants and children when paediatric formulations are not available is challenging and in many cases halves of adult tablets are administered, leading to inaccurate dosing. As mentioned before, an additional sub-group analysis revealed that 35% of participants with virological failure had been prescribed low doses of antiretrovirals at least once. Of note, after the One Stop Clinic was established in Ifakara, such dosing errors did not occur again. Moreover, the younger they are, the more children rely on a diligent caregiver to adhere to ART.

Compared to PI-based regimens, children and adolescents on NNRTI-based regimens had much higher odds to experience virological failure and acquire drug resistance mutations. This difference between groups suggests good performance of PI regimens in suppressing HIV and preventing the development of resistance mutations. However, the additional counselling that patients switched to second line receive may have biased these results.

Girls were more vulnerable to virological failure and the development of mutations for reasons that we could not explain in the framework of this study, but indeed sex inequalities were also found in other recent studies from East Africa (Lazzerini *et al.*, 2016).

Our results confirmed the already described very low sensitivity of the WHO immunological and clinical criteria to detect treatment failure (Mutwa *et al.*, 2014). Not having routine viral load monitoring leads to an unacceptable delay in the diagnosis of failure and accumulation of mutations, and limits treatment options (Sutcliffe *et al.*, 2008; Sigaloff *et al.*, 2011, 2012).

After a decade of successful ART rollout in Africa, children and adolescents still represent an underprivileged population. ART coverage is lower and failure rates are higher than in adults. The management of treatment failure is still suboptimal in resource-limited settings due to limited availability of drug choices, limited access to viral load, and a general lack of guidance in national treatment guidelines, with consequent delays in switching from failing regimens (Davies *et al.*, 2011). Awareness needs to be raised in order to advocate for the strengthening of adherence strategies tailored to this vulnerable population, the development and widespread availability of new paediatric ART formulations and the universal roll-out of routine viral load monitoring for the millions of children and adolescents living with HIV.

10.3. The way forward: implications for policy and practice

The findings of this thesis have implications for policy and practice to ensure a better care for HIV-infected pregnant women, mothers and children. These implications are relevant for Ifakara and similar rural settings across sub-Saharan Africa.

Some of the results can and should be translated into immediate actions at a public health and service delivery levels.

- ✓ *Integration of antenatal, postpartum and PMTCT/HIV services*. Offering HIV testing, ART and clinical follow-up at the reproductive and child health clinics is key to identify HIV-infected

mothers, facilitate their linkage and retention in care and provide the necessary care to HIV-exposed infants.

- ✓ *Continuous counselling throughout pregnancy and breastfeeding periods*. Women diagnosed to be HIV-infected during pregnancy must receive continuous counselling. Post-test counselling does improve linkage to HIV care but is not sufficient. Education and counselling of women and, ideally, their male partners have to be incorporated as part of the routine care delivered during clinic visits to enhance retention and adherence to ART.

- ✓ *Early Infant Diagnosis strengthening.* In order to ensure effective EID circuits in the priority Global Plan countries, decentralised laboratory capacity to perform pro-viral HIV DNA PCR is necessary at a distrital, or at least regional level. Laboratory capacity has to be coupled with effective modes to deliver results to the health facilities and strategies to track mother-infant pairs.

- ✓ *Provider-initiated HIV testing and counselling*. Opt-out HIV testing has to be effectively incorporated as routine practice in all health facility settings. Offering also an HIV test to partners and children of index cases will further increase the benefits of provider-initiated HIV testing. This approach needs to be well articulated to ensure that patients' rights are respected and HIV testing is not coercive.

- ✓ *Family and child-centred units*. Family and child-friendly units or clinic days can be easily organized within functioning HIV clinics. They have the potential to improve the care and retention of children and families. HIV clinics in sub-Saharan Africa should incorporate such approach and adapt it to the size of the paediatric population they attend.

- ✓ *Expansion of routine viral load monitoring*. The findings of this thesis contribute to the already significant evidence to advocate for the implementation of viral load monitoring in all settings, and specifically for the treatment monitoring of children. Timely detection of treatment failure is crucial to improve clinical outcomes, preserve treatment options and prevent transmission of HIV infection.

Priority research questions have emerged from this PhD work. Despite the changes introduced on the service delivery model for HIV-affected families in Ifakara, some gaps persist. Further research is needed to tackle these questions and adapt PMTCT and paediatric HIV programs in rural African settings.

- *Strengthening of HIV re-screening in late pregnancy and HIV testing in the labour ward*. To maximise the coverage of PMTCT it is crucial to identify women who have seroconverted during pregnancy and test those who reach the labour with an unknown serostatus. Continuous education of the attending staff and a reliable supply chain of tests are essential to bridge these gaps.

- *"Test and start" or better "test, ensure readiness and start"?* Early disengagement from care of HIV-infected pregnant women is the main downside of Option B+. This has been partially attributed to the immediate ART initiation policy. Despite it is clear that an accelerated ART initiation should be recommended, different timings in the context of Option B+ need to be studied to ensure the optimal treatment coverage, adherence and retention in care.

- *HIV testing outside health facilities*. Approaches to expand HIV testing outside health facilities are not yet fully explored. Interventions involving the community, schools and religious and spiritual congregations can contribute to overcome HIV testing barriers related to stigma and health access.

- *Development of paediatric drugs*. The development of new antiretroviral drugs and appropriate formulations for children continues to be far too slow. Few paediatric antiretroviral formulations and fixed-dose combinations are available. This represents an important limitation for paediatric ART programs and undermines children's adherence. Health agencies, pharmaceutical companies and donors must joint efforts to ensure that treatment is available for all children in need.

In conclusion, ART scaling-up for pregnant women and children in sub-Saharan Africa should be based on integrated service delivery models; the expansion of case-finding strategies to identify HIV-infected children; the widespread implementation of routine viral load monitoring; the use of point-of-care, or at least locally-based, EID tests; and the adoption of tailored interventions to enhance adherence and retention among pregnant women, children and adolescents.

11. Conclusions

Study 1

1. Several gaps were identified through the PMTCT care pathway in Ifakara. The most important ones were: a) no re-testing of seronegative pregnant women in late pregnancy; b) poor linkage of newly-diagnosed HIV-infected pregnant women into HIV care; and c) lack of a standardized follow-up of HIV-exposed infants.
2. The prior complexity of the guidelines, with different recommendations and antiretroviral regimens based on the immunological and clinical stage of pregnant women, and the infant's feeding method, prevented a broad and efficient PMTCT coverage.
3. The lack of integration and comprehensiveness of the different health services that HIV-affected pregnant women and mother-infant pairs require further aggravated the low uptake of PMTCT recommendations.

Study 2

4. The implementation of the One Stop Clinic of Ifakara combined with the improved efficiency of a paperless clinic and the rollout of provider initiated HIV testing and counselling and EID resulted in: a) an increased number of pregnant women and children diagnosed and linked into care; b) an increased detection of children with AIDS; c) universal ART coverage; d) lower loss to follow-up and better ascertainment of mortality; and e) a proof that elimination of MTCT can be achieved in rural Tanzania.
5. Children enrolled after 2012 presented with more advance disease and immunosuppression, reflecting the high yield of infants and children enrolled from the in-patient wards. Measures to identify HIV-infected children must be implemented in parallel to the efforts to achieve universal PMTCT and the elimination of MTCT.
6. The One Stop Clinic model may provide a feasible and scalable model for delivering high-quality family-centred HIV care in Tanzania and other sub-Saharan African countries.

Study 3

7. The implementation of Option B+ through an integrated service delivery model resulted in: a) universal HIV testing in the antenatal care clinic; b) high rates of linkage into care and ART prescription; and c) a MTCT rate below the elimination threshold.

8. HIV re-screening in late pregnancy and HIV testing during labour were still poorly implemented, preventing mother-infant pairs with high risk of MTCT from receiving an appropriate PMTCT intervention.
9. Losses along the PMTCT cascade were concentrated among newly-diagnosed HIV-infected pregnant women and during the first months after diagnosis. Intensified counselling after diagnosis and specific interventions for tracking women lost during the first months should be implemented.

Study 4

10. The absence of routine viral load monitoring lead to a late recognition of children with treatment failure and development of multi-class HIV drug resistance-associated mutations that threatened the efficacy of the second line drugs available in Tanzania.

Study 5

11. A high rate of virological failure (25.4%) was observed among 213 HIV-infected children and adolescents after a median of 4.5 years on ART. This rate exceeded by far the 9.1% observed for the adult population in the same cohort. The prevalence of drug resistance mutations in children and adolescents with virological failure was 90.4% and 79% of these patients had multi-class resistances.
12. Both virological failure and the emergence of drug resistance-associated mutations were associated with NNRTI use, younger age at ART initiation, poor adherence, and female gender.
13. The implementation and scale-up of viral load monitoring is essential to maintain treatment options and optimize health outcomes in resource-limited setting with restricted treatment possibilities. New drug classes and new paediatric formulations are urgently needed in sub-Saharan Africa to be able to treat individuals with multi-class resistant virus.

12. List of references

Abrams, E. J. *et al.* (1998) 'Association of human immunodeficiency virus (HIV) load early in life with disease progression among HIV-infected infants. New York City Perinatal HIV Transmission Collaborative Study Group', *The Journal of Infectious Diseases*, 178(1), pp. 101–108.

Ahmed, S. *et al.* (2013) 'Beyond early infant diagnosis: case finding strategies for identification of HIV-infected infants and children', *AIDS (London, England)*, 27 Suppl 2, pp. S235-245. doi: 10.1097/QAD.0000000000000099.

Ahmed, S. *et al.* (2016) 'Lost opportunities to identify and treat HIV-positive patients: results from a baseline assessment of provider-initiated HIV testing and counselling (PITC) in Malawi', *Tropical medicine & international health: TM & IH*, 21(4), pp. 479–485. doi: 10.1111/tmi.12671.

Aliyu, M. H. *et al.* (2016) 'Integrated prevention of mother-to-child HIV transmission services, antiretroviral therapy initiation, and maternal and infant retention in care in rural north-central Nigeria: a cluster-randomised controlled trial', *The lancet. HIV*, 3(5), pp. e202-211. doi: 10.1016/S2352-3018(16)00018-7.

Ambia, J. and Mandala, J. (2016) 'A systematic review of interventions to improve prevention of mother-to-child HIV transmission service delivery and promote retention', *Journal of the International AIDS Society*, 19(1), p. 20309.

Anaky, M.-F. *et al.* (2010) 'Scaling up antiretroviral therapy for HIV-infected children in Côte d'Ivoire: determinants of survival and loss to programme', *Bulletin of the World Health Organization*, 88(7), pp. 490–499. doi: 10.2471/BLT.09.068015.

Azcoaga-Lorenzo, A. *et al.* (2011) 'Effectiveness of a PMTCT programme in rural Western Kenya', *AIDS care*, 23(3), pp. 274–280. doi: 10.1080/09540121.2010.507750.

Badri, M. *et al.* (2006) 'Cost-effectiveness of highly active antiretroviral therapy in South Africa', *PLoS medicine*, 3(1), p. e4. doi: 10.1371/journal.pmed.0030004.

Bangsberg, D. R., Moss, A. R. and Deeks, S. G. (2004) 'Paradoxes of adherence and drug resistance to HIV antiretroviral therapy', *The Journal of Antimicrobial Chemotherapy*, 53(5), pp. 696–699. doi: 10.1093/jac/dkh162.

Barker, P. M. *et al.* (2007) 'Strategies for the scale-up of antiretroviral therapy in South Africa through health system optimization', *The Journal of Infectious Diseases*, 196 Suppl 3, pp. S457-463. doi: 10.1086/521110.

Barker, P. M., Mphatswe, W. and Rollins, N. (2011) 'Antiretroviral drugs in the cupboard are not enough: the impact of health systems' performance on mother-to-child transmission of HIV', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 56(2), pp. e45-48. doi: 10.1097/QAI.0b013e3181fdbf20.

Beltman, J. J. *et al.* (2010) 'Accelerated HIV testing for PMTCT in maternity and labour wards is vital to capture mothers at a critical point in the programme at district level in Malawi', *AIDS care*, 22(11), pp. 1367–1372. doi: 10.1080/09540121003758473.

Bernays, S. *et al.* (2014) 'Children growing up with HIV infection: the responsibility of success', *Lancet (London, England)*, 383(9925), pp. 1355–1357. doi: 10.1016/S0140-6736(13)62328-4.

Bikaako-Kajura, W. *et al.* (2006) 'Disclosure of HIV status and adherence to daily drug regimens among HIV-infected children in Uganda', *AIDS and behavior*, 10(4 Suppl), pp. S85-93. doi: 10.1007/s10461-006-9141-3.

Bolton-Moore, C. *et al.* (2007) 'Clinical outcomes and CD4 cell response in children receiving antiretroviral therapy at primary health care facilities in Zambia', *JAMA*, 298(16), pp. 1888–1899. doi: 10.1001/jama.298.16.1888.

Bong, C.-N. *et al.* (2007) 'Risk factors for early mortality in children on adult fixed-dose combination antiretroviral treatment in a central hospital in Malawi', *AIDS (London, England)*, 21(13), pp. 1805–1810. doi: 10.1097/QAD.0b013e3282c3a9e4.

Both, J. M. C. and van Roosmalen, J. (2010) 'The impact of Prevention of Mother to Child Transmission (PMTCT) programmes on maternal health care in resource-poor settings: looking beyond the PMTCT programme--a systematic review', *BJOG: an international journal of obstetrics and gynaecology*, 117(12), pp. 1444–1450. doi: 10.1111/j.1471-0528.2010.02692.x.

Braitstein, P. *et al.* (2010) 'Retention of HIV-infected and HIV-exposed children in a comprehensive HIV clinical care programme in Western Kenya', *Tropical medicine & international health: TM & IH*, 15(7), pp. 833–841. doi: 10.1111/j.1365-3156.2010.02539.x.

Bratholm, C. *et al.* (2010) 'Drug resistance is widespread among children who receive long-term antiretroviral treatment at a rural Tanzanian hospital', *The Journal of Antimicrobial Chemotherapy*, 65(9), pp. 1996–2000. doi: 10.1093/jac/dkq234.

de Bruyn, M. and Paxton, S. (2005) 'HIV testing of pregnant women--what is needed to protect positive women's needs and rights?', *Sexual Health*, 2(3), pp. 143–151.

Buck, W. C. *et al.* (2013) 'Risk factors for mortality in Malawian children with human immunodeficiency virus and tuberculosis co-infection', *The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease*, 17(11), pp. 1389–1395. doi: 10.5588/ijtld.13.0030.

Busza, J. *et al.* (2014) '"I don't want financial support but verbal support." How do caregivers manage children's access to and retention in HIV care in urban Zimbabwe?', *Journal of the International AIDS Society*, 17, p. 18839.

Buzdugan, R. *et al.* (2012) 'Positive attitudes to pediatric HIV testing: findings from a nationally representative survey from Zimbabwe', *PloS One*, 7(12), p. e53213. doi: 10.1371/journal.pone.0053213.

Centers for Disease Control and Prevention (CDC) (2013) 'Impact of an innovative approach to prevent mother-to-child transmission of HIV--Malawi, July 2011-September 2012', *MMWR. Morbidity and mortality weekly report*, 62(8), pp. 148–151.

Centers for Disease Control (CDC) (1981) 'Pneumocystis pneumonia--Los Angeles', *MMWR. Morbidity and mortality weekly report*, 30(21), pp. 250–252.

Chamla, D. *et al.* (2013) 'Evidence from the field: missed opportunities for identifying and linking HIV-infected children for early initiation of ART', *AIDS (London, England)*, 27 Suppl 2, pp. S139-146. doi: 10.1097/QAD.0000000000000101.

Chan, A. K. *et al.* (2016) 'Same day HIV diagnosis and antiretroviral therapy initiation affects retention in Option B+ prevention of mother-to-child transmission services at antenatal care in Zomba District, Malawi', *Journal of the International AIDS Society*, 19(1), p. 20672.

Chatterjee, A. *et al.* (2011) 'Implementing services for Early Infant Diagnosis (EID) of HIV: a comparative descriptive analysis of national programs in four countries', *BMC public health*, 11, p. 553. doi: 10.1186/1471-2458-11-553.

Chetty, T. *et al.* (2012) 'A retrospective study of Human Immunodeficiency Virus transmission, mortality and loss to follow-up among infants in the first 18 months of life in a prevention of mother-to-child transmission programme in an urban hospital in KwaZulu-Natal, South Africa', *BMC pediatrics*, 12, p. 146. doi: 10.1186/1471-2431-12-146.

Ciaranello, A. L. *et al.* (2009) 'Effectiveness of pediatric antiretroviral therapy in resource-limited settings: a systematic review and meta-analysis', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 49(12), pp. 1915–1927. doi: 10.1086/648079.

Ciaranello, A. L. *et al.* (2012) 'What will it take to eliminate pediatric HIV? Reaching WHO target rates of mother-to-child HIV transmission in Zimbabwe: a model-based analysis', *PLoS medicine*, 9(1), p. e1001156. doi: 10.1371/journal.pmed.1001156.

Ciaranello, A. L. *et al.* (2013) 'Cost-effectiveness of World Health Organization 2010 guidelines for prevention of mother-to-child HIV transmission in Zimbabwe', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 56(3), pp. 430–446. doi: 10.1093/cid/cis858.

Cohen, D., Lungu, M. and van Oosterhout, J. J. (2010) 'HIV testing coverage of family members of adult antiretroviral therapy patients in Malawi', *AIDS care*, 22(11), pp. 1346–1349. doi: 10.1080/09540121003720986.

Cohen, M. S. *et al.* (2011) 'Prevention of HIV-1 infection with early antiretroviral therapy', *The New England Journal of Medicine*, 365(6), pp. 493–505. doi: 10.1056/NEJMoa1105243.

Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Study Group *et al.* (2008) 'Response to combination antiretroviral therapy: variation by age', *AIDS (London, England)*, 22(12), pp. 1463–1473. doi: 10.1097/QAD.0b013e3282f88d02.

Connor, E. M. *et al.* (1994) 'Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group', *The New England Journal of Medicine*, 331(18), pp. 1173–1180. doi: 10.1056/NEJM199411033311801.

Cook, R. E. *et al.* (2011) 'Predictors of successful early infant diagnosis of HIV in a rural district hospital in Zambézia, Mozambique', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 56(4), pp. e104-109. doi: 10.1097/QAI.0b013e318207a535.

Cotton, M. F. *et al.* (2013) 'Early time-limited antiretroviral therapy versus deferred therapy in South African infants infected with HIV: results from the children with HIV early antiretroviral (CHER) randomised trial', *Lancet (London, England)*, 382(9904), pp. 1555–1563. doi: 10.1016/S0140-6736(13)61409-9.

Davies, M.-A. *et al.* (2011) 'Virologic failure and second-line antiretroviral therapy in children in South Africa--the IeDEA Southern Africa collaboration', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 56(3), pp. 270–278. doi: 10.1097/QAI.0b013e3182060610.

De Cock, K. M. *et al.* (2000) 'Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice', *JAMA*, 283(9), pp. 1175–1182.

De Cock, K. M. and El-Sadr, W. M. (2013) 'When to start ART in Africa--an urgent research priority', *The New England Journal of Medicine*, 368(10), pp. 886–889. doi: 10.1056/NEJMp1300458.

DNDi (2013) 'Refocusing research and development of paediatric HIV treatments on the needs of children in resource-poor settings: a paediatric HIV roundtable with industry & joint call to action on paediatric HIV. Dakar, Senegal; October 24, 2013. Hosted by DNDi'. Available at: http://www.dndi.org/images/stories/diseases_portfolio/DNDi_Paediatric_HIV_roundtable.pdf (Accessed: 13 March 2017).

Doherty, T. M., McCoy, D. and Donohue, S. (2005) 'Health system constraints to optimal coverage of the prevention of mother-to-child HIV transmission programme in South Africa: lessons from the implementation of the national pilot programme', *African Health Sciences*, 5(3), pp. 213–218. doi: 10.5555/afhs.2005.5.3.213.

Drake, A. L. *et al.* (2014) 'Incident HIV during pregnancy and postpartum and risk of mother-to-child HIV transmission: a systematic review and meta-analysis', *PLoS medicine*, 11(2), p. e1001608. doi: 10.1371/journal.pmed.1001608.

EACS (2011) 'European AIDS Clinical Society Guidelines (2011)'. Available at: http://www.eacsociety.org/files/2011_eacsguidelines-v6.0-english_oct.pdf (Accessed: 6 March 2017).

Ebuy, H., Yebyo, H. and Alemayehu, M. (2015) 'Level of adherence and predictors of adherence to the Option B+ PMTCT programme in Tigray, northern Ethiopia', *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*, 33, pp. 123–129. doi: 10.1016/j.ijid.2014.12.026.

Eley, B. *et al.* (2006) 'Antiretroviral treatment for children', *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde*, 96(9 Pt 2), pp. 988–993.

Ellis, J. C. *et al.* (2007) 'Nevirapine concentrations in HIV-infected children treated with divided fixed-dose combination antiretroviral tablets in Malawi and Zambia', *Antiviral Therapy*, 12(2), pp. 253–260.

Emmett, S. D. *et al.* (2010) 'Predicting virologic failure among HIV-1-infected children receiving antiretroviral therapy in Tanzania: a cross-sectional study', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 54(4), pp. 368–375. doi: 10.1097/QAI.0b013e3181cf4882.

Erb, S. *et al.* (2017) 'Health care provider communication training in rural Tanzania empowers HIV-infected patients on antiretroviral therapy to discuss adherence problems', *HIV medicine*. doi: 10.1111/hiv.12499.

Erb, S et al. (2015) 'Healthcare provider communication training program improves adherence assessment in HIV-infected patients treated with antiretroviral therapy (ART) in rural Tanzania. 15th European AIDS Conference; Barcelona, Spain; October 21-24, 2015. Abstract BPD1/3.' *15th European AIDS Conference*, Barcelona, Spain, 21 October.

Essajee, S. *et al.* (2017) 'Scale-up of Early Infant HIV Diagnosis and Improving Access to Pediatric HIV Care in Global Plan Countries: Past and Future Perspectives', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 75 Suppl 1, pp. S51–S58. doi: 10.1097/QAI.0000000000001319.

Essajee, S. M. *et al.* (2013) 'Pediatric treatment 2.0: ensuring a holistic response to caring for HIV-exposed and infected children', *AIDS (London, England)*, 27 Suppl 2, pp. S215-224. doi: 10.1097/QAD.0000000000000091.

European Collaborative Study (2005) 'Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 40(3), pp. 458–465. doi: 10.1086/427287.

Fergusson, P. and Tomkins, A. (2009) 'HIV prevalence and mortality among children undergoing treatment for severe acute malnutrition in sub-Saharan Africa: a systematic review and meta-analysis', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103(6), pp. 541–548. doi: 10.1016/j.trstmh.2008.10.029.

Ferrand, R. A. *et al.* (2016) 'Implementation and Operational Research: The Effectiveness of Routine Opt-Out HIV Testing for Children in Harare, Zimbabwe', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 71(1), pp. e24-29. doi: 10.1097/QAI.0000000000000867.

Fetzer, B. C. *et al.* (2011) 'Barriers to and facilitators of adherence to pediatric antiretroviral therapy in a sub-Saharan setting: insights from a qualitative study', *AIDS patient care and STDs*, 25(10), pp. 611–621. doi: 10.1089/apc.2011.0083.

Fillekes, Q. *et al.* (2011) 'Pediatric underdosing of efavirenz: a pharmacokinetic study in Uganda', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 58(4), pp. 392–398. doi: 10.1097/QAI.0b013e318235e560.

Foster, G. *et al.* (2017) 'Impact of Facility-Based Mother Support Groups on Retention in Care and PMTCT Outcomes in Rural Zimbabwe: The EPAZ Cluster-Randomized Controlled Trial', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 75 Suppl 2, pp. S207–S215. doi: 10.1097/QAI.0000000000001360.

Gamell, A. *et al.* (2013) 'Uptake of guidelines on prevention of mother-to-child transmission of HIV in rural Tanzania: time for change', *Swiss Medical Weekly*, 143, p. w13775. doi: 10.4414/smw.2013.13775.

Gamell, A., Muri, L., *et al.* (2016) 'A Case Series of Acquired Drug Resistance-Associated Mutations in Human Immunodeficiency Virus-Infected Children: An Emerging Public Health Concern in Rural Africa', *Open Forum Infectious Diseases*, 3(1), p. ofv199. doi: 10.1093/ofid/ofv199.

Gamell, A., Glass, T. R., *et al.* (2016) 'Implementation and Operational Research: An Integrated and Comprehensive Service Delivery Model to Improve Pediatric and Maternal HIV Care in Rural Africa', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 73(5), pp. e67–e75. doi: 10.1097/QAI.0000000000001178.

Gamell, A et al. (2015a) 'The One Stop Clinic of Ifakara: a model for integration of prevention of mother-to-child transmission and pediatric HIV services. 9th Conference of Tropical Medicine and International Health; Basel, Switzerland; September 6-10, 2015'. *9th Conference of Tropical Medicine and International Health*, Basel, Switzerland, 6 September.

Gamell, A et al. (2015b) 'The One Stop Clinic of Ifakara: implementing a bundle of measures for paediatric HIV services in rural Africa to reach the UNAIDS' target "90-90-90". 15th European AIDS Conference; Barcelona, Spain; October 21-24, 2015.' *15th European AIDS Conference*, Barcelona, Spain, 21 October.

Garcia, P. M. *et al.* (1999) 'Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group', *The New England Journal of Medicine*, 341(6), pp. 394–402. doi: 10.1056/NEJM199908053410602.

Gebrekristos, H. T., Mlisana, K. P. and Karim, Q. A. (2005) 'Patients' readiness to start highly active antiretroviral treatment for HIV', *BMJ (Clinical research ed.)*, 331(7519), pp. 772–775. doi: 10.1136/bmj.331.7519.772.

Geddes, R. *et al.* (2011) 'Dual and triple therapy to prevent mother-to-child transmission of HIV in a resource-limited setting - lessons from a South African programme', *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde*, 101(9), pp. 651–654.

Geng, E. H. *et al.* (2015) 'Estimation of Mortality among HIV-infected people on antiretroviral therapy treatment in east Africa: a sampling based approach in an observational, multisite, cohort study', *The lancet. HIV*, 2(3), pp. e107–e116. doi: 10.1016/S2352-3018(15)00002-8.

Gray, R. H. *et al.* (2005) 'Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study', *Lancet (London, England)*, 366(9492), pp. 1182–1188. doi: 10.1016/S0140-6736(05)67481-8.

Green, A. *et al.* (2014) 'Clinical mentorship of nurse initiated antiretroviral therapy in Khayelitsha, South Africa: a quality of care assessment', *PloS One*, 9(6), p. e98389. doi: 10.1371/journal.pone.0098389.

Gupta, N. *et al.* (2013) 'Clinical outcomes of a comprehensive integrated program for HIV-exposed infants: a 3-year experience promoting HIV-free survival in rural Rwanda', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 62(4), pp. e109-114. doi: 10.1097/QAI.0b013e31827d5118.

Haas, A. D. *et al.* (2016) 'Retention in care during the first 3 years of antiretroviral therapy for women in Malawi's option B+ programme: an observational cohort study', *The lancet. HIV*, 3(4), pp. e175-182. doi: 10.1016/S2352-3018(16)00008-4.

Haraka, F. *et al.* (2015) 'A Bundle of Services Increased Ascertainment of Tuberculosis among HIV-Infected Individuals Enrolled in a HIV Cohort in Rural Sub-Saharan Africa', *PloS One*, 10(4), p. e0123275. doi: 10.1371/journal.pone.0123275.

Hesseling, A. C. *et al.* (2009) 'High incidence of tuberculosis among HIV-infected infants: evidence from a South African population-based study highlights the need for improved tuberculosis control strategies', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 48(1), pp. 108–114. doi: 10.1086/595012.

Horwood, C. *et al.* (2010) 'Routine checks for HIV in children attending primary health care facilities in South Africa: attitudes of nurses and child caregivers', *Social Science & Medicine (1982)*, 70(2), pp. 313–320. doi: 10.1016/j.socscimed.2009.10.002.

Hsiao, N.-Y., Stinson, K. and Myer, L. (2013) 'Linkage of HIV-infected infants from diagnosis to antiretroviral therapy services across the Western Cape, South Africa', *PloS One*, 8(2), p. e55308. doi: 10.1371/journal.pone.0055308.

ICAP (2006) 'Pediatric antiretroviral dosing in resource-limited settings. International Center for AIDS Care and Treatment Programs (ICAP), Columbia University Mailman School of Public Health, New York, NY, USA; 2006'. Available at: http://www.cdc.gov/globalaids/docs/program-areas/pmtct/peds-dosing-guide-8.5-x-11-122006.pdf (Accessed: 13 March 2017).

INSIGHT START Study Group *et al.* (2015) 'Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection', *The New England Journal of Medicine*, 373(9), pp. 795–807. doi: 10.1056/NEJMoa1506816.

Kamya, M. R. *et al.* (2007) 'Predictors of long-term viral failure among ugandan children and adults treated with antiretroviral therapy', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 46(2), pp. 187–193. doi: 10.1097/QAI.0b013e31814278c0.

Kankasa, C. *et al.* (2009) 'Routine offering of HIV testing to hospitalized pediatric patients at university teaching hospital, Lusaka, Zambia: acceptability and feasibility', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 51(2), pp. 202–208.

Kantor, R. *et al.* (2009) 'Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 49(3), pp. 454–462. doi: 10.1086/600396.

Kasenga, F., Hurtig, A.-K. and Emmelin, M. (2007) 'Home deliveries: implications for adherence to nevirapine in a PMTCT programme in rural Malawi', *AIDS care*, 19(5), pp. 646–652. doi: 10.1080/09540120701235651.

Kebe, K. *et al.* (2013) 'High rate of antiretroviral drug resistance mutations in HIV type 1-infected Senegalese children in virological failure on first-line treatment according to the World Health Organization guidelines', *AIDS research and human retroviruses*, 29(2), pp. 242–249. doi: 10.1089/aid.2011.0300.

Kellerman, S. E. and Sugandhi, N. (2013) 'Pediatric AIDS in the elimination agenda', *PLoS medicine*, 10(8), p. e1001503. doi: 10.1371/journal.pmed.1001503.

Kim, M. H. *et al.* (2015) 'Implementation and operational research: the impact of option B+ on the antenatal PMTCT cascade in Lilongwe, Malawi', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 68(5), pp. e77-83. doi: 10.1097/QAI.0000000000000517.

Kirsten, I. *et al.* (2011) 'Adherence to combination prophylaxis for prevention of mother-to-child-transmission of HIV in Tanzania', *PloS One*, 6(6), p. e21020. doi: 10.1371/journal.pone.0021020.

Kline, M. W. *et al.* (2009) 'The Pediatric AIDS Corps: responding to the African HIV/AIDS health professional resource crisis', *Pediatrics*, 123(1), pp. 134–136. doi: 10.1542/peds.2008-0402.

Koller, M. *et al.* (2015) 'Immunodeficiency in children starting antiretroviral therapy in low-, middle-, and high-income countries', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 68(1), pp. 62–72. doi: 10.1097/QAI.0000000000000380.

Koole, O. *et al.* (2014) 'Improved retention of patients starting antiretroviral treatment in Karonga District, northern Malawi, 2005-2012', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 67(1), pp. e27-33. doi: 10.1097/QAI.0000000000000252.

Kranzer, K. *et al.* (2014) 'Barriers to provider-initiated testing and counselling for children in a high HIV prevalence setting: a mixed methods study', *PLoS medicine*, 11(5), p. e1001649. doi: 10.1371/journal.pmed.1001649.

Kurewa, E. N. *et al.* (2011) 'Realities and Challenges of a Five Year Follow Up of Mother and Child Pairs on a PMTCT Program in Zimbabwe', *The open AIDS journal*, 5, pp. 51–58. doi: 10.2174/1874613601105010051.

Lahuerta, M. *et al.* (2014) 'Advanced HIV disease at entry into HIV care and initiation of antiretroviral therapy during 2006-2011: findings from four sub-saharan African countries', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 58(3), pp. 432–441. doi: 10.1093/cid/cit724.

Lazzerini, M. *et al.* (2016) 'Mortality and its risk factors in Malawian children admitted to hospital with clinical pneumonia, 2001-12: a retrospective observational study', *The Lancet. Global Health*, 4(1), pp. e57-68. doi: 10.1016/S2214-109X(15)00215-6.

Le, T. *et al.* (2013) 'Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy', *The New England Journal of Medicine*, 368(3), pp. 218–230. doi: 10.1056/NEJMoa1110187.

Letang, E. *et al.* (2015) 'Cryptococcal Antigenemia in Immunocompromised Human Immunodeficiency Virus Patients in Rural Tanzania: A Preventable Cause of Early Mortality', *Open Forum Infectious Diseases*, 2(2), p. ofv046. doi: 10.1093/ofid/ofv046.

Letang, E. *et al.* (2017) 'Cohort profile: The Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) - A prospective HIV cohort in rural Tanzania', *Swiss Medical Weekly*, 147, p. w14485. doi: smw.2017.14485.

van Lettow, M. *et al.* (2014) 'Towards elimination of mother-to-child transmission of HIV: performance of different models of care for initiating lifelong antiretroviral therapy for pregnant women in Malawi (Option B+)', *Journal of the International AIDS Society*, 17, p. 18994.

Leyenaar, J. K. *et al.* (2010) 'Early clinical outcomes in children enrolled in human immunodeficiency virus infection care and treatment in lesotho', *The Pediatric Infectious Disease Journal*, 29(4), pp. 340–345. doi: 10.1097/INF.0b013e3181bf8ecb.

Lindegren, M. L. *et al.* (2012) 'Integration of HIV/AIDS services with maternal, neonatal and child health, nutrition, and family planning services', *The Cochrane Database of Systematic Reviews*, (9), p. CD010119. doi: 10.1002/14651858.CD010119.

Lowenthal, E. D. *et al.* (2014) 'Perinatally acquired HIV infection in adolescents from sub-Saharan Africa: a review of emerging challenges', *The Lancet. Infectious Diseases*, 14(7), pp. 627–639. doi: 10.1016/S1473-3099(13)70363-3.

Lu, L et al. (2011) 'HIV incidence in women during the first postpartum year: implications for PMTCT programs Francistown, Botswana, 2010. 3rd International Workshop on HIV Pediatrics; Rome, Italy; 2011'. *3rd International Workshop on HIV Pediatrics*, Rome, Italy.

Luyirika, E. *et al.* (2013) 'Scaling up paediatric HIV care with an integrated, family-centred approach: an observational case study from Uganda', *PloS One*, 8(8), p. e69548. doi: 10.1371/journal.pone.0069548.

Masimba, P. *et al.* (2013) 'Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania', *AIDS research and human retroviruses*, 29(9), pp. 1229–1236. doi: 10.1089/AID.2011.0367.

McCollum, E. D. *et al.* (2010) 'Task shifting routine inpatient pediatric HIV testing improves program outcomes in urban Malawi: a retrospective observational study', *PloS One*, 5(3), p. e9626. doi: 10.1371/journal.pone.0009626.

McCollum, E. D. *et al.* (2011) 'Routine inpatient human immunodeficiency virus testing system increases access to pediatric human immunodeficiency virus care in sub-Saharan Africa', *The Pediatric Infectious Disease Journal*, 30(5), pp. e75-81. doi: 10.1097/INF.0b013e3182103f8a.

McConnell, M. S. *et al.* (2010) 'National program scale-up and patient outcomes in a pediatric antiretroviral treatment program, Thailand, 2000-2007', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 54(4), pp. 423–429. doi: 10.1097/QAI.0b013e3181dc5eb0.

Medley, A. *et al.* (2004) 'Rates, barriers and outcomes of HIV serostatus disclosure among women in developing countries: implications for prevention of mother-to-child transmission programmes', *Bulletin of the World Health Organization*, 82(4), pp. 299–307.

Menson, E. N. *et al.* (2006) 'Underdosing of antiretrovirals in UK and Irish children with HIV as an example of problems in prescribing medicines to children, 1997-2005: cohort study', *BMJ (Clinical research ed.)*, 332(7551), pp. 1183–1187. doi: 10.1136/bmj.332.7551.1183.

Mirkuzie, A. H. *et al.* (2011) 'Current status of medication adherence and infant follow up in the prevention of mother to child HIV transmission programme in Addis Ababa: a cohort study', *Journal of the International AIDS Society*, 14, p. 50. doi: 10.1186/1758-2652-14-50.

Mirkuzie, A. H., Hinderaker, S. G. and Mørkve, O. (2010) 'Promising outcomes of a national programme for the prevention of Mother-to-Child HIV transmission in Addis Ababa: a retrospective study', *BMC health services research*, 10, p. 267. doi: 10.1186/1472-6963-10-267.

Morgan, D. and Whitworth, J. (2001) 'The natural history of HIV-1 infection in Africa', *Nature Medicine*, 7(2), pp. 143–145. doi: 10.1038/84564.

Moses, S. E. *et al.* (2008) 'HIV-1 seroconversion during pregnancy resulting in vertical transmission', *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 41(2), pp. 152–153. doi: 10.1016/j.jcv.2007.10.013.

Mossdorf, E. *et al.* (2011) 'Improved antiretroviral treatment outcome in a rural African setting is associated with cART initiation at higher CD4 cell counts and better general health condition', *BMC infectious diseases*, 11, p. 98. doi: 10.1186/1471-2334-11-98.

Mugasha, C. *et al.* (2014) 'Intra-facility linkage of HIV-positive mothers and HIV-exposed babies into HIV chronic care: rural and urban experience in a resource limited setting', *PloS One*, 9(12), p. e115171. doi: 10.1371/journal.pone.0115171.

Munthali, T. *et al.* (2015) 'Mortality and morbidity patterns in under-five children with severe acute malnutrition (SAM) in Zambia: a five-year retrospective review of hospital-based records (2009-2013)', *Archives of Public Health = Archives Belges De Sante Publique*, 73(1), p. 23. doi: 10.1186/s13690-015-0072-1.

Muri, L. *et al.* (2016) 'Development of HIV drug resistance and therapeutic failure in children and adolescents in rural tanzania - an emerging public health concern', *AIDS (London, England)*. doi: 10.1097/QAD.0000000000001273.

Mutanga, J. N. *et al.* (2012) 'Institutionalizing provider-initiated HIV testing and counselling for children: an observational case study from Zambia', *PloS One*, 7(4), p. e29656. doi: 10.1371/journal.pone.0029656.

Mutevedzi, P. C. and Newell, M.-L. (2014) 'Review: [corrected] The changing face of the HIV epidemic in sub-Saharan Africa', *Tropical medicine & international health: TM & IH*, 19(9), pp. 1015–1028. doi: 10.1111/tmi.12344.

Mutwa, P. R. *et al.* (2014) 'Long-term effectiveness of combination antiretroviral therapy and prevalence of HIV drug resistance in HIV-1-infected children and adolescents in Rwanda', *The Pediatric Infectious Disease Journal*, 33(1), pp. 63–69. doi: 10.1097/INF.0b013e31829e6b9f.

Myer, L. and Akugizibwe, P. (2009) 'Impact of HIV treatment scale-up on women's reproductive health care and reproductive rights in Southern Africa', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 52 Suppl 1, pp. S52-53. doi: 10.1097/QAI.0b013e3181bbc818.

Myer, L. and Phillips, T. K. (2017) 'Beyond "Option B+": Understanding Antiretroviral Therapy (ART) Adherence, Retention in Care and Engagement in ART Services Among Pregnant and Postpartum Women Initiating Therapy in Sub-Saharan Africa', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 75 Suppl 2, pp. S115–S122. doi: 10.1097/QAI.0000000000001343.

Nachega, J. B. *et al.* (2009) 'Antiretroviral therapy adherence, virologic and immunologic outcomes in adolescents compared with adults in southern Africa', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 51(1), pp. 65–71. doi: 10.1097/QAI.0b013e318199072e.

Newell, M.-L. *et al.* (2004) 'Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis', *Lancet (London, England)*, 364(9441), pp. 1236–1243. doi: 10.1016/S0140-6736(04)17140-7.

Nkonki, L. L. *et al.* (2007) 'Missed opportunities for participation in prevention of mother to child transmission programmes: simplicity of nevirapine does not necessarily lead to optimal uptake, a qualitative study', *AIDS research and therapy*, 4, p. 27. doi: 10.1186/1742-6405-4-27.

Nouhin, J. *et al.* (2013) 'Increased risk of Q151M and K65R mutations in patients failing stavudine-containing first-line antiretroviral therapy in Cambodia', *PloS One*, 8(8), p. e73744. doi: 10.1371/journal.pone.0073744.

Ntamatungiro, A. J. *et al.* (2017) 'Strengthening HIV therapy and care in rural Tanzania affects rates of viral suppression', *The Journal of Antimicrobial Chemotherapy*. doi: 10.1093/jac/dkx095.

Nuwagaba-Biribonwoha, H. *et al.* (2010) 'Introducing a multi-site program for early diagnosis of HIV infection among HIV-exposed infants in Tanzania', *BMC pediatrics*, 10, p. 44. doi: 10.1186/1471-2431-10-44.

Nyandiko, W. M. *et al.* (2010) 'Outcomes of HIV-exposed children in western Kenya: efficacy of prevention of mother to child transmission in a resource-constrained setting', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 54(1), pp. 42–50. doi: 10.1097/QAI.0b013e3181d8ad51.

Ononge, S. *et al.* (2014) 'Predictors of unknown HIV serostatus at the time of labor and delivery in Kampala, Uganda', *International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics*, 124(3), pp. 235–239. doi: 10.1016/j.ijgo.2013.08.011.

van Oosterhout, J. J. G. *et al.* (2009) 'Diagnosis of antiretroviral therapy failure in Malawi: poor performance of clinical and immunological WHO criteria', *Tropical medicine & international health: TM & IH*, 14(8), pp. 856–861. doi: 10.1111/j.1365-3156.2009.02309.x.

Pasquet, A. *et al.* (2010) 'Impact of drug stock-outs on death and retention to care among HIV-infected patients on combination antiretroviral therapy in Abidjan, Côte d'Ivoire', *PloS One*, 5(10), p. e13414. doi: 10.1371/journal.pone.0013414.

Patel, K. *et al.* (2008) 'Long-term effects of highly active antiretroviral therapy on CD4+ cell evolution among children and adolescents infected with HIV: 5 years and counting', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 46(11), pp. 1751–1760. doi: 10.1086/587900.

Paton, N. I. *et al.* (2014) 'Assessment of second-line antiretroviral regimens for HIV therapy in Africa', *The New England Journal of Medicine*, 371(3), pp. 234–247. doi: 10.1056/NEJMoa1311274.

Penazzato, M. *et al.* (2014) 'Task shifting for the delivery of pediatric antiretroviral treatment: a systematic review', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 65(4), pp. 414–422. doi: 10.1097/QAI.0000000000000024.

Penazzato, M. *et al.* (2015) 'Optimizing drugs to reach treatment targets for children and adolescents living with HIV', *Journal of the International AIDS Society*, 18(Suppl 6), p. 20270.

Penazzato, M. *et al.* (2017) 'Pediatric Treatment Scale-Up: The Unfinished Agenda of the Global Plan', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 75 Suppl 1, pp. S59–S65. doi: 10.1097/QAI.0000000000001333.

Preidis, G. A. *et al.* (2013) 'Routine inpatient provider-initiated HIV testing in Malawi, compared with client-initiated community-based testing, identifies younger children at higher risk of early mortality', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 63(1), pp. e16-22. doi: 10.1097/QAI.0b013e318288aad6.

Puthanakit, T. *et al.* (2007) 'Hospitalization and mortality among HIV-infected children after receiving highly active antiretroviral therapy', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 44(4), pp. 599–604. doi: 10.1086/510489.

Ramírez-Mena, A. *et al.* (2016) 'Prevalence and Outcomes of Hepatitis B Coinfection and Associated Liver Disease Among Antiretroviral Therapy-Naive Individuals in a Rural Tanzanian Human Immunodeficiency Virus Cohort', *Open Forum Infectious Diseases*, 3(3), p. ofw162. doi: 10.1093/ofid/ofw162.

Reddi, A. *et al.* (2007) 'Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa', *BMC pediatrics*, 7, p. 13. doi: 10.1186/1471-2431-7-13.

Reynolds, S. J. and Quinn, T. C. (2010) 'Setting the stage: current state of affairs and major challenges', *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 50 Suppl 3, pp. S71-76. doi: 10.1086/651476.

Rochat, T. J. *et al.* (2011) 'Towards a family-centered approach to HIV treatment and care for HIV-exposed children, their mothers and their families in poorly resourced settings', *Future Virology*, 6(6), pp. 687–696. doi: 10.2217/fvl.11.45.

van Rossum, A. M. C., Fraaij, P. L. A. and de Groot, R. (2002) 'Efficacy of highly active antiretroviral therapy in HIV-1 infected children', *The Lancet. Infectious Diseases*, 2(2), pp. 93–102.

Saleem, H., Kyeyagalire, R. and Lunsford, S. S. (2014) 'Patient and provider perspectives on improving the linkage of HIV-positive pregnant women to long-term HIV care and treatment in eastern Uganda', *African journal of AIDS research: AJAR*, 13(1), pp. 45–51. doi: 10.2989/16085906.2014.892015.

Salou, M. *et al.* (2016) 'High rates of virological failure and drug resistance in perinatally HIV-1-infected children and adolescents receiving lifelong antiretroviral therapy in routine clinics in Togo', *Journal of the International AIDS Society*, 19(1), p. 20683.

Schouten, E. J. *et al.* (2011) 'Prevention of mother-to-child transmission of HIV and the health-related Millennium Development Goals: time for a public health approach', *Lancet (London, England)*, 378(9787), pp. 282–284. doi: 10.1016/S0140-6736(10)62303-3.

SECOND-LINE Study Group *et al.* (2013) 'Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study', *Lancet (London, England)*, 381(9883), pp. 2091–2099. doi: 10.1016/S0140-6736(13)61164-2.

Severe, P. *et al.* (2010) 'Early versus standard antiretroviral therapy for HIV-infected adults in Haiti', *The New England Journal of Medicine*, 363(3), pp. 257–265. doi: 10.1056/NEJMoa0910370.

Shelton, J. D. (2011) 'HIV/AIDS. ARVs as HIV prevention: a tough road to wide impact', *Science (New York, N.Y.)*, 334(6063), pp. 1645–1646. doi: 10.1126/science.1212353.

Shroufi, A. *et al.* (2013) 'Mother to Mother (M2M) peer support for women in Prevention of Mother to Child Transmission (PMTCT) programmes: a qualitative study', *PloS One*, 8(6), p. e64717. doi: 10.1371/journal.pone.0064717.

Sibanda, E. L. *et al.* (2013) 'The magnitude of loss to follow-up of HIV-exposed infants along the prevention of mother-to-child HIV transmission continuum of care: a systematic review and meta-analysis', *AIDS (London, England)*, 27(17), pp. 2787–2797. doi: 10.1097/QAD.0000000000000027.

Sigaloff, K. C. E. *et al.* (2011) 'HIV-1-resistance-associated mutations after failure of first-line antiretroviral treatment among children in resource-poor regions: a systematic review', *The Lancet. Infectious Diseases*, 11(10), pp. 769–779. doi: 10.1016/S1473-3099(11)70141-4.

Sigaloff, K. C. E. *et al.* (2012) 'Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa', *AIDS research and human retroviruses*, 28(2), pp. 171–175. doi: 10.1089/aid.2011.0136.

Simoni, J. M. *et al.* (2007) 'Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management', *Pediatrics*, 119(6), pp. e1371-1383. doi: 10.1542/peds.2006-1232.

Soorapanth, S. *et al.* (2006) 'Cost-effectiveness of HIV rescreening during late pregnancy to prevent mother-to-child HIV transmission in South Africa and other resource-limited settings', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 42(2), pp. 213–221. doi: 10.1097/01.qai.0000214812.72916.bc.

Stoeckle, M. *et al.* (2006) 'Moving up from 3 by 5', *The Lancet. Infectious Diseases*, 6(8), pp. 460–461. doi: 10.1016/S1473-3099(06)70529-1.

Sutcliffe, C. G. *et al.* (2008) 'Effectiveness of antiretroviral therapy among HIV-infected children in sub-Saharan Africa', *The Lancet. Infectious Diseases*, 8(8), pp. 477–489. doi: 10.1016/S1473-3099(08)70180-4.

Tanzanian MoHSW (2007a) 'Guidelines for HIV testing and counselling in clinical settings. National AIDS Control Programme. Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; 2007'. Available at: http://ihi.eprints.org/823/1/MoHSW.pdf_(53).pdf (Accessed: 13 March 2017).

Tanzanian MoHSW (2007b) 'Prevention of mother-to-child transmission of HIV. National guidelines. Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; 2007'. Available at: http://apps.who.int/medicinedocs/documents/s19272en/s19272en.pdf (Accessed: 6 March 2017).

Tanzanian MoHSW (2012) 'National guidelines for comprehensive care of prevention of mother-to-child transmission of HIV services. Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; 2012'. Available at: http://ihi.eprints.org/3337/1/PMTCT.pdf (Accessed: 6 March 2017).

Tanzanian MoHSW (2013a) 'Implementation of HIV/AIDS care and treatment services in Tanzania. National AIDS Control Programme. Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; 2013'. Available at: www.nacp.go.tz/site/download/bookreport3.pdf (Accessed: 13 March 2017).

Tanzanian MoHSW (2013b) 'National guidelines for comprehensive care services for prevention of mother-to-child transmission of HIV and keeping their mothers alive. Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania; 2013'. Available at: http://pmtct.or.tz/wp-content/uploads/2013/10/tz_guidelines_ccs_optionb_all.pdf (Accessed: 1 December 2016).

Tanzanian MoHSW (2014) 'Global AIDS response country progress report: United republic of Tanzania. Ministry of Health and Social Wellfare, dar es Salaam, United Republic of Tanzania; 2014'. Available at: http://www.unaids.org/sites/default/files/country/documents/TZA_narrative_report_2014.pdf (Accessed: 10 March 2017).

Tanzanian MoHSW (2015) 'National guidelines for the management of HIV and AIDS. National AIDS control programme, Ministry of health and social welfare, dar es Salaam, United Republic of Tanzania, 2015'. Available at: https://aidsfree.usaid.gov/sites/default/files/04_11_2016.tanzania_national_guideline_for_management_hiv_and_aids_may_2015._tagged.pdf (Accessed: 18 January 2017).

Tanzanian NBS (2011) 'Tanzania Demographic and Health Survey 2010. National Bureau of Statistics and ICF Macro, Dar es Salaam, United Republic of Tanzania; 2011'. Available at: http://dhsprogram.com/pubs/pdf/FR243/FR243%5B24June2011%5D.pdf (Accessed: 18 January 2017).

Tanzanian NBS (2012) 'Population and housing census 2012, Morogoro region. National Bureau of Statistics, Ministry of Finances, Dar es Salaam, United Republic of Tanzania; 2012'. Available at: http://www.nbs.go.tz/nbstz/index.php/english/statistics-by-subject/population-and-housing-census/697-2012-phc-regional-profiles (Accessed: 18 January 2017).

Technau, K.-G. *et al.* (2014) 'Timing of maternal HIV testing and uptake of prevention of mother-to-child transmission interventions among women and their infected infants in Johannesburg, South Africa', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 65(5), pp. e170-178. doi: 10.1097/QAI.0000000000000068.

TEMPRANO ANRS 12136 Study Group *et al.* (2015) 'A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa', *The New England Journal of Medicine*, 373(9), pp. 808–822. doi: 10.1056/NEJMoa1507198.

TenoRes Study Group (2016) 'Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study', *The Lancet. Infectious Diseases*, 16(5), pp. 565–575. doi: 10.1016/S1473-3099(15)00536-8.

Tenthani, L. *et al.* (2012) 'Involving expert patients in antiretroviral treatment provision in a tertiary referral hospital HIV clinic in Malawi', *BMC health services research*, 12, p. 140. doi: 10.1186/1472-6963-12-140.

Tenthani, L. *et al.* (2014) 'Retention in care under universal antiretroviral therapy for HIV-infected pregnant and breastfeeding women ('Option B+') in Malawi', *AIDS (London, England)*, 28(4), pp. 589–598. doi: 10.1097/QAD.0000000000000143.

Tonwe-Gold, B. *et al.* (2009) 'Implementing family-focused HIV care and treatment: the first 2 years' experience of the mother-to-child transmission-plus program in Abidjan, Côte d'Ivoire', *Tropical medicine & international health: TM & IH*, 14(2), pp. 204–212. doi: 10.1111/j.1365-3156.2008.02182.x.

Tweya, H. *et al.* (2014) 'Understanding factors, outcomes and reasons for loss to follow-up among women in Option B+ PMTCT programme in Lilongwe, Malawi', *Tropical medicine & international health: TM & IH*, 19(11), pp. 1360–1366. doi: 10.1111/tmi.12369.

UNAIDS (2010a) 'Country fact sheets. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2010'. Available at: http://www.unaids.org/en/dataanalysis/tools/aidsinfo/countryfactsheets/ (Accessed: 25 January 2013).

UNAIDS (2010b) 'UNAIDS report on the global AIDS epidemic 2010. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2010'. Available at: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf (Accessed: 18 January 2017).

UNAIDS (2011) '2011-2015: Global plan towards the elimination of new HIV infections among children by 2015 and keeping their mothers alive. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2011'. Available at: http://files.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20110609_JC2137_Global-Plan-Elimination-HIV-Children_en.pdf (Accessed: 30 April 2017).

UNAIDS (2013) 'UNAIDS report on the global AIDS epidemic 2013. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2013'. Available at: http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf (Accessed: 10 March 2017).

UNAIDS (2014) '90-90-90 An ambitious treatment target to help end the AIDS epidemic. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2014'. Available at: http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf (Accessed: 10 March 2017).

UNAIDS (2014) '2014 Progress report on the global plan towards the elimination of new HIV infections among children by 2015 and keeping their mothers alive. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2014'. Available at: http://www.unaids.org/sites/default/files/documents/JC2681_2014-Global-Plan-progress_en.pdf (Accessed: 10 March 2017).

UNAIDS (2014) 'The Gap Report. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2014'. Available at: http://files.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf (Accessed: 20 March 2017).

UNAIDS (2015a) '2015 Progress report on the global plan towards the elimination of new HIV infections among children and keeping their mothers alive. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; 2015'. Available at: http://www.unaids.org/sites/default/files/media_asset/JC2774_2015ProgressReport_GlobalPlan_en.pdf (Accessed: 18 January 2017).

UNAIDS (2015b) 'Country factsheets: United Republic of Tanzania. AIDSinfo, Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2015'. Available at: http://aidsinfo.unaids.org/ (Accessed: 29 April 2017).

UNAIDS (2016a) '2016 Global AIDS update. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2016'. Available at: http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_en.pdf (Accessed: 30 April 2017).

UNAIDS (2016b) 'Children and HIV. Factsheet July 2016. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2016'. Available at: http://www.unaids.org/sites/default/files/media_asset/FactSheet_Children_en.pdf (Accessed: 18 January 2017).

UNAIDS (2017) 'Latest statistics on the status of the AIDS epidemic. Factsheet July 2017. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; 2017'. Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf (Accessed: 26 August 2017).

Vanobberghen, F. *et al.* (2017) 'A decade of HIV care in rural Tanzania: Trends in clinical outcomes and impact of clinic optimisation in an open, prospective cohort', *PloS One*, 12(7), p. e0180983. doi: 10.1371/journal.pone.0180983.

Vanobberghen, F et al. (2015a) 'A decade of HIV care and treatment in rural Tanzania: trends in treatment, opportunistic infections and laboratory abnormalities among HIV-positive adults. 15th European AIDS Conference; Barcelona, Spain; October 21-24, 2015. Abstract PE21/5'. *15th European AIDS Conference*, Barcelona, Spain, 21 October.

Vanobberghen, F et al. (2015b) 'Challenges in estimating death and retention rates in a longitudinal cohort of HIV-infected persons in rural Tanzania. 9th European Conference on Tropical Medicine and International Health; Basel, Switzerland; September 6-10, 2015'. *th*

European Conference on Tropical Medicine and International Health, Basel, Switzerland, 6 September.

Violari, A. *et al.* (2008) 'Early antiretroviral therapy and mortality among HIV-infected infants', *The New England Journal of Medicine*, 359(21), pp. 2233–2244. doi: 10.1056/NEJMoa0800971.

Vreeman, R. C. *et al.* (2008) 'A systematic review of pediatric adherence to antiretroviral therapy in low- and middle-income countries', *The Pediatric Infectious Disease Journal*, 27(8), pp. 686–691. doi: 10.1097/INF.0b013e31816dd325.

Walker, A. S. *et al.* (2004) 'Response to highly active antiretroviral therapy varies with age: the UK and Ireland Collaborative HIV Paediatric Study', *AIDS (London, England)*, 18(14), pp. 1915–1924.

Walmsley, S. *et al.* (2002) 'Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection', *The New England Journal of Medicine*, 346(26), pp. 2039–2046. doi: 10.1056/NEJMoa012354.

Wamalwa, D. C. *et al.* (2013) 'Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy', *Journal of Acquired Immune Deficiency Syndromes (1999)*, 62(3), pp. 267–274. doi: 10.1097/QAI.0b013e31827b4ac8.

Ward, J et al. (2014) 'Surveillance of acquired HIV drug resitances among children in the national ART program: pilot cross-sectional surveys at sentinel ART sites. Tanzania Comission for AIDS (TACAIDS) HIV Research Forum; Dar es Salaam, tanzania; October 2014.' *Tanzania Comission for AIDS (TACAIDS) HIV Research Forum*, Dar es Salaam, tanzania, October.

Weigel, R. *et al.* (2009) 'Effect of provider-initiated testing and counselling and integration of ART services on access to HIV diagnosis and treatment for children in Lilongwe, Malawi: a pre-post comparison', *BMC pediatrics*, 9, p. 80. doi: 10.1186/1471-2431-9-80.

When To Start Consortium *et al.* (2009) 'Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies', *Lancet (London, England)*, 373(9672), pp. 1352–1363. doi: 10.1016/S0140-6736(09)60612-7.

WHO (2004) 'Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. World Health Organization, Geneva, Switzerland; 2004'. Available at: http://www.who.int/hiv/pub/mtct/en/arvdrugswomenguidelinesfinal.pdf?ua=1 (Accessed: 18 January 2017).

WHO (2007a) 'Guidance on provider-initiated HIV testing and counselling in health facilities. World Health Organization, Geneva, Switzerland; 2007'. Available at: http://apps.who.int/iris/bitstream/10665/43688/1/9789241595568_eng.pdf (Accessed: 26 May 2017).

WHO (2007b) 'Update based on the technical consultation held on behalf of the Inter-agency Task Team (IATT) on prevention of hIV infection in pregnant women, mothers and their infants. World Health Organization, Geneva, Switzerlans; October 2006'. Available at:

http://apps.who.int/iris/bitstream/10665/43747/1/9789241595964_eng.pdf (Accessed: 10 March 2017).

WHO (2010a) 'Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. Worl health Organization, Geneva, Switzerland; 2010'. Available at: http://apps.who.int/iris/bitstream/10665/75236/1/9789241599818_eng.pdf (Accessed: 6 March 2017).

WHO (2010b) 'Antiretroviral therapy for HIV infection in infants and children: towards universal access. Recommendations for a public health approach, 2010 revision. World Health Organization, Geneva, Switzerland; 2010'. Available at: http://apps.who.int/iris/bitstream/10665/164255/1/9789241599801_eng.pdf?ua=1 (Accessed: 20 March 2017).

WHO (2010c) 'Towards universal access. Scaling up priority HIV/AIDS interventions in the health sector. Progress report 2010. World Health Organization, Geneva, Switzerland; 2010'. Available at: http://www.who.int/hiv/pub/2010progressreport/summary_en.pdf?ua=1 (Accessed: 6 March 2017).

WHO (2010d) 'WHO recommendations on the diagnosis of HIV infection in infants and children. World Health Organization, Geneva, Switzerland; 2010'. Available at: http://apps.who.int/iris/bitstream/10665/44275/1/9789241599085_eng.pdf (Accessed: 29 April 2017).

WHO (2011) 'Global HIV/AIDS response. Epidemic update and health sector progress towards universal access. Progress report 2011. World Health Organization, Geneva, Switzerland; 2011'. Available at: http://apps.who.int/iris/bitstream/10665/44787/1/9789241502986_eng.pdf (Accessed: 10 March 2017).

WHO (2012a) 'Programmatic update: Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. World Health Organization, Geneva, Switzerland; 2012'. Available at: http://www.who.int/hiv/PMTCT_update.pdf (Accessed: 6 March 2017).

WHO (2012b) 'Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. World Health Organization, Geneva, Switzerland; 2012'. Available at: http://apps.who.int/iris/bitstream/10665/70892/2/WHO_HIV_2012.6_eng.pdf (Accessed: 18 January 2017).

WHO (2013) 'Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. World Health Organization, geneva, Switzerland; 2013'. Available at: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf (Accessed: 13 March 2017).

WHO (2014a) 'Global guidance on criteria and processes for validation: elimination of mother-to-child transmission of HIV and syphilis. World Health Organization, Geneva, Switzerland; 2014'. Available at:

http://apps.who.int/iris/bitstream/10665/112858/1/9789241505888_eng.pdf?ua=1&ua=1 (Accessed: 18 January 2017).

WHO (2014b) 'The global health sector strategy on HIV/AIDS 2011-2015: an interim review of progress, May 2014. World health Organization, Geneva, Switzerland; 2014'. Available at: http://apps.who.int/iris/bitstream/10665/112790/1/9789241507295_eng.pdf (Accessed: 10 March 2017).

WHO (2015) 'Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. World Health Organization, Geneva, Switzerland; 2015'. Available at: http://apps.who.int/iris/bitstream/10665/186275/1/9789241509565_eng.pdf?ua=1 (Accessed: 1 May 2017).

WHO (2016) 'Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. World Health Organization, Geneva, Switzerland; 2016'. Available at: http://apps.who.int/iris/bitstream/10665/208825/1/9789241549684_eng.pdf?ua=1 (Accessed: 18 January 2017).

Wilkinson, L. S. *et al.* (2015) 'Self-transfer and mortality amongst adults lost to follow-up in ART programmes in low- and middle-income countries: systematic review and meta-analysis', *Tropical medicine & international health: TM & IH*, 20(3), pp. 365–379. doi: 10.1111/tmi.12434.

Wolfe, B. A. *et al.* (2006) 'The OpenMRS system: collaborating toward an open source EMR for developing countries', *AMIA ... Annual Symposium proceedings. AMIA Symposium*, p. 1146.

Wudineh, F. and Damtew, B. (2016) 'Mother-to-Child Transmission of HIV Infection and Its Determinants among Exposed Infants on Care and Follow-Up in Dire Dawa City, Eastern Ethiopia', *AIDS research and treatment*, 2016, p. 3262746. doi: 10.1155/2016/3262746.

Yeap, A. D. *et al.* (2010) 'Factors influencing uptake of HIV care and treatment among children in South Africa - a qualitative study of caregivers and clinic staff', *AIDS care*, 22(9), pp. 1101–1107. doi: 10.1080/09540121003602218.

Youngleson, M. S. *et al.* (2010) 'Improving a mother to child HIV transmission programme through health system redesign: quality improvement, protocol adjustment and resource addition', *PloS One*, 5(11), p. e13891. doi: 10.1371/journal.pone.0013891.

Zoufaly, A. *et al.* (2013) 'Prevalence and determinants of virological failure in HIV-infected children on antiretroviral therapy in rural Cameroon: a cross-sectional study', *Antiviral Therapy*, 18(5), pp. 681–690. doi: 10.3851/IMP2562.